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Anti-TNF antibodies and methotrexate in the treatment of autoimmune disease

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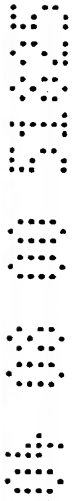(71) Applicant(s)
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Anti-TNF Antibodies and Methotrexate in the treatment of Autoimmune Disease

Abstract

Methods for treating and/or preventing a TNF-mediated disease in an individual are
disclosed. Also disclosed is a composition comprising methotrexate and an anti-tumour
necrosis factor antibody. TNF-mediated diseases include rheumatoid arthritis, Crohn's
disease and acute and chronic immune diseases associated with transplantation.

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ORIGINAL

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Invention Title:	Anti-TNF Antibodies and Methotrexate in the Treatment of Autoimmune Disease

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

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ANTI-TNF ANTIBODIES AND METHOTREXATE
IN THE TREATMENT OF AUTOIMMUNE DISEASE

Description

Background of the Invention

Monocytes and macrophages secrete cytokines known as tumor necrosis factor alpha (TNFα) and tumor necrosis factor beta (TNFβ) in response to endotoxin or other stimuli. TNFα is a soluble homotrimer of 17 kD protein subunits (Smith et al., *J. Biol. Chem.* 262:6951- 6954 (1987)). A membrane-bound 26 kD precursor form of TNF also exists (Kriegler et al., *Cell* 53:45-53 (1988)). For reviews of TNF, see Beutler et al., *Nature* 320:584 (1986); Old, *Science* 230:630 (1986); and Le et al., *Lab. Invest.* 56:234 (1987).

Cells other than monocytes or macrophages also produce TNFα. For example, human non-monocytic tumor cell lines produce tumor necrosis factor (TNF) (Rubin et al., *J. Exp. Med.* 164:1350 (1986); Spriggs et al., *Proc. Natl. Acad. Sci. USA* 84:6563 (1987)). CD4+ and CD8+ peripheral blood T

lymphocytes and some cultured T and B cell lines (Cuturi et al., *J. Exp. Med.* 165:1581 (1987); Sung et al., *J. Exp. Med.* 168:1539 (1988); Turner et al., *Eur. J. Immunol.* 17:1807-1814 (1987)) also produce TNFα.

5   TNF causes pro-inflammatory actions which result in tissue injury, such as degradation of cartilage and bone, induction of adhesion molecules, inducing procoagulant activity on vascular endothelial cells (Pober et al., *J. Immunol.* 136:1680 (1986)), increasing the adherence of

10   neutrophils and lymphocytes (Pober et al., *J. Immunol.* 138:3319 (1987)), and stimulating the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi et al., *J. Exp. Med.* 166:1390 (1987)).

15   Recent evidence associates TNF with infections (Cerami et al., *Immunol. Today* 9:28 (1988)), immune disorders, neoplastic pathologies (Oliff et al., *Cell* 50:555 (1987)), autoimmune pathologies and graft-versus-host pathologies (Piguet et al., *J. Exp. Med.* 166:1280 (1987)). The

20   association of TNF with cancer and infectious pathologies is often related to the host's catabolic state. Cancer patients suffer from weight loss, usually associated with anorexia.

        The extensive wasting which is associated with cancer,

25   and other diseases, is known as "cachexia" (Kern et al., *J. Parent. Enter. Nutr.* 12:286-298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of body mass in response to a malignant growth. The fundamental physiological derangement can relate to a

30   decline in food intake relative to energy expenditure. The cachectic state causes most cancer morbidity and mortality. TNF can mediate cachexia in cancer, infectious pathology, and other catabolic states.

        TNF also plays a central role in gram-negative sepsis

35   and endotoxic shock (Michie et al., *Br. J. Surg.* 76:670-671

(1989); Debets et al., *Second Vienna Shock Forum*. p.463-466
(1989); Simpson et al., *Crit. Care Clin.* 5:27-47 (1989)),
including fever, malaise, anorexia, and cachexia.
Endotoxin strongly activates monocyte/macrophage production
5 and secretion of TNF and other cytokines (Kornbluth et al.,
J. Immunol. 137:2585-2591 (1986)). TNF and other
monocyte-derived cytokines mediate the metabolic and
neurohormonal responses to endotoxin (Michie et al., *New
Engl. J. Med.* 318:1481-1486 (1988)). Endotoxin
10 administration to human volunteers produces acute illness
with flu-like symptoms including fever, tachycardia,
increased metabolic rate and stress hormone release
(Revhaug et al., *Arch. Surg.* 123:162-170 (1988)).
Circulating TNF increases in patients suffering from
15 Gram-negative sepsis (Waage et al., *Lancet* 1:355-357
(1987); Hammerle et al., *Second Vienna Shock Forum*
p. 715-718 (1989); Debets et al., *Crit. Care Med.*
17:489-497 (1989); Calandra et al., *J. Infect. Dis.*
161:982-987 (1990)).
20   Thus, TNFα has been implicated in inflammatory
diseases, autoimmune diseases, viral, bacterial and
parasitic infections, malignancies, and/or neurogenerative
diseases and is a useful target for specific biological
therapy in diseases, such as rheumatoid arthritis and
25 Crohn's disease. Beneficial effects in open-label trials
with a chimeric monoclonal antibody to TNFα (cA2) have been
reported with suppression of inflammation (Elliott et al.,
Arthritis Rheum. 36:1681-1690 (1993); Elliott et al.,
Lancet 344:1125-1127 (1994)). See also, Van Dullemen et
30 al., *Gastroenterology* 109:129-135 (1995). Beneficial
results in a randomized, double-blind, placebo-controlled
trial with cA2 have also been reported with suppression of
inflammation (Elliott et al., *Lancet* 344:1105-1110 (1994)).

Summary of the Invention

The present invention is based on the discovery that treatment of patients suffering from a TNF-mediated disease with a tumor necrosis factor antagonist, such as an anti-
5 tumor necrosis factor antibody, as adjunctive and/or concomitant therapy to methotrexate therapy produces a rapid and sustained reduction in the clinical signs and symptoms of the disease. The present invention is also based on the unexpected and dramatic discovery that a
10 multiple dose regimen of a tumor necrosis factor antagonist, such as an anti-tumor necrosis factor antibody, when administered adjunctively with methotrexate to an individual suffering from a TNF-mediated disease produces a highly beneficial or synergistic clinical response for a
15 significantly longer duration compared to that obtained with a single or multiple dose regimen of the antagonist administered alone or that obtained with methotrexate administered alone. As a result of Applicants' invention, a method is provided herein for treating and/or preventing
20 a TNF-mediated disease in an individual comprising co-administering an anti-TNF antibody or a fragment thereof and methotrexate to the individual in therapeutically effective amounts. In a particular embodiment, methotrexate is administered in the form of a series of low
25 doses separated by intervals of days or weeks.

A method is also provided herein for treating and/or preventing recurrence of a TNF-mediated disease in an individual comprising co-administering an anti-TNF antibody or a fragment thereof and methotrexate to the individual in
30 therapeutically effective amounts. TNF-mediated diseases include rheumatoid arthritis, Crohn's disease, and acute and chronic immune diseases associated with an allogenic transplantation (e.g., renal, cardiac, bone marrow, liver, pancreatic, small intestine, skin or lung transplantation).

Therefore, in one embodiment, the invention relates to a method of treating and/or preventing rheumatoid arthritis in an individual comprising co-administering an anti-TNF antibody or a fragment thereof and methotrexate to the
5 individual in therapeutically effective amounts. In a second embodiment, the invention relates to a method of treating and/or preventing Crohn's disease in an individual comprising co-administering an anti-TNF antibody or a fragment thereof and methotrexate to the individual in
10 therapeutically effective amounts. In a third embodiment, the invention relates to a method of treating and/or preventing other autoimmune diseases and/or acute or chronic immune disease associated with a transplantation in an individual, comprising co-administering an anti-TNF
15 antibody or a fragment thereof and methotrexate to the individual in therapeutically effective amounts.

A further embodiment of the invention relates to compositions comprising an anti-TNF antibody or a fragment thereof and methotrexate.
20 In addition to anti-TNF antibodies, TNF antagonists include anti-TNF antibodies and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase
25 inhibitors (e.g. pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; and compounds which prevent and/or inhibit TNF receptor signalling.

Brief Description of the Drawings
30 Figures 1A-1C are a set of three graphs showing the results over time for swollen joint count in rheumatoid arthritis (RA) patients receiving cA2 treatment (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without methotrexate. Results for the placebo group (methotrexate alone) are shown with

the 1 mg/kg group. The number of patients with data at each evaluation visit is shown at the bottom of each graph. White circle = - methotrexate (MTX-); black circle = + methotrexate (MTX+); square = placebo.

5   Figures 2A-2C are a set of three graphs showing the results over time for tender joint count in RA patients receiving cA2 treatment (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without methotrexate. Results for the placebo group (methotrexate alone) are shown with the 1 mg/kg group. The
10 number of patients with data at each evaluation visit is shown at the bottom of each graph. White circle = - methotrexate; black circle = + methotrexate; square = placebo.

     Figures 3A-3C are a set of three graphs showing the
15 results over time for the Physician's Global Disease Assessment in RA patients receiving cA2 treatment (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without methotrexate. Results for the placebo group (methotrexate alone) are shown with the 1 mg/kg group. The number of patients with data at
20 each evaluation visit is shown at the bottom of each graph. White circle = - methotrexate; black circle = + methotrexate; square = placebo.

     Figures 4A-4C are a set of three graphs showing the results over time for the Patient Disease Assessment in RA
25 patients receiving cA2 treatment (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without methotrexate. Results for the placebo group (methotrexate alone) are shown with the 1 mg/kg group. The number of patients with data at each evaluation visit is shown at the bottom of each graph.
30 White circle = - methotrexate; black circle = + methotrexate; square = placebo.

     Figures 5A-5C are a set of three graphs showing the results over time for C-reactive protein (CRP) concentration in RA patients receiving cA2 treatment
35 (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without

methotrexate. Results for the placebo group (methotrexate alone) are shown with the 1 mg/kg group. The number of patients with data at each evaluation visit is shown at the bottom of each graph. White circle = - methotrexate; black
5 circle = + methotrexate; square = placebo.

Figures 6A-6C are a set of three graphs showing the results over time for the Health Assessment Questionnaire (HAQ) in RA patients receiving cA2 treatment (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without methotrexate. Results
10 for the placebo group (methotrexate alone) are shown with the 1 mg/kg group. The number of patients with data at each evaluation visit is shown at the bottom of each graph. White circle = - methotrexate; black circle = + methotrexate; square = placebo.

15 Figures 7A-7F are a set of six graphs showing the serum cA2 concentration in each RA patient receiving cA2 treatment (1 mg/kg, 3 mg/kg or 10 mg/kg) with or without methotrexate, plotted over time. Data plotted are the serum cA2 concentrations obtained just before the
20 administration of cA2 at weeks 2, 6, 10 and 14 and then at weeks 18 and 26. The scales for the serum cA2 concentration are condensed with higher doses of cA2.

Figures 8A and 8B are a set of two graphs showing the median serum cA2 concentration over time in RA patients
25 receiving 3 mg/kg cA2 (top panel) or 10 mg/kg cA2 (bottom panel) with or without methotrexate. Square = + methotrexate; circle or triangle = - methotrexate.

Detailed Description of the Invention

The present invention relates to the discovery that
30 tumor necrosis factor antagonists can be administered to patients suffering from a TNF-mediated disease as adjunctive and/or concomitant therapy to methotrexate therapy, with good to excellent alleviation of the signs and symptoms of the disease. The present invention also

relates to the discovery that tumor necrosis factor antagonists can be administered to patients suffering from a TNF-mediated disease in multiple doses and as adjunctive and/or concomitant therapy to methotrexate therapy, with a

5 significant improvement in duration of clinical response.

As a result of Applicants' invention, a method is provided herein for treating and/or preventing a TNF-mediated disease in an individual, comprising co-administering methotrexate and a tumor necrosis factor

10 antagonist to the individual in therapeutically effective amounts. The TNF antagonist and methotrexate can be administered simultaneously or sequentially. The TNF antagonist and methotrexate can each be administered in single or multiple doses. Multiple TNF antagonists can be

15 co-administered with methotrexate. Other therapeutic regimens and agents can be used in combination with the therapeutic co-administration of TNF antagonists and methotrexate or other drugs that suppress the immune system.

20 A method is also provided herein for treating and/or preventing recurrence of a TNF-mediated disease in an individual comprising co-administering methotrexate and a TNF antagonist to the individual in therapeutically effective amounts.

25 As used herein, a "TNF-mediated disease" refers to a TNF related pathology or disease. TNF related pathologies or diseases include, but are not limited to, the following:

(A) acute and chronic immune and autoimmune pathologies, such as, but not limited to, rheumatoid

30 arthritis (RA), juvenile chronic arthritis (JCA), thyroiditis, graft versus host disease (GVHD), scleroderma, diabetes mellitus, Graves' disease, allergy, acute or chronic immune disease associated with an allogenic transplantation, such as, but not limited to, renal

35 transplantation, cardiac transplantation, bone marrow

transplantation, liver transplantation, pancreatic transplantation, small intestine transplantation, lung transplantation and skin transplantation;

(B) infections, including, but not limited to, sepsis
5 syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, acute and chronic parasitic and/or infectious diseases, bacterial, viral or fungal, such as a human innunodeficiency virus (HIV), acquired immunodeficiency
10 syndrome (AIDS) (including symptoms of cachexia, autoimmune disorders, AIDS dementia complex and infections);

(C) inflammatory diseases, such as chronic inflammatory pathologies, including chronic inflammatcry pathologies such as, but not limited to, sarcoidosis,
15 chronic inflammatory bowel disease, ulcerative colitis, and Crohn's pathology or disease; vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, Kawasaki's pathology and vasculitis syndromes, such as, but not
20 limited to, polyarteritis nodosa, Wegener's granulomatosis, Henoch-Schönlein purpura, giant cell arthritis and micrcscopic vasculitis of the kidneys; chronic active hepatitis; Sjögren's syndrome; spondyloarthropathies, such as ankylosing spondylitis, psoriatic arthritis and
25 spondylitis, enteropathic arthritis and spondylitis, reactive arthritis and arthritis associated with inflammatory bowel disease; and uveitis;

(D) neurodegenerative diseases, including, but not limited to, demyelinating diseases, such as multiple
30 sclerosis and acute transverse myelitis; myasthenia gravis; extrapyramidal and cerebellar disorders, such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders, such as Huntington's chorea and senile chorea; drug-induced
35 movement disorders, such as those induced by drugs which

block central nervous system (CNS) dopamine receptors;
hypokinetic movement disorders, such as Parkinson's
disease; progressive supranucleo palsy; cerebellar and
spinocerebellar disorders, such as astructural lesions of
5   the cerebellum; spinocerebellar degenerations (spinal
ataxia, Friedreich's ataxia, cerebellar cortical
degenerations, multiple systems degenerations (Mencel,
Dejerine-Thomas, Shi-Drager, and MachadoJoseph)); and
systemic disorders (Refsum's disease, abetalipoprotemia,
10 ataxia, telangiectasia, and mitochondrial multisystem
disorder); disorders of the motor unit, such as neurogenic
muscular atrophies (anterior horn cell degeneration, such
as amyotrophic lateral sclerosis, infantile spinal muscular
atrophy and juvenile spinal muscular atrophy); Alzheimer's
15 disease; Down's syndrome in middle age; diffuse Lewy body
disease; senile dementia of Lewy body type;
Wernicke-Korsakoff syndrome; chronic alcoholism; primary
biliary cirrhosis; cryptogenic fibrosing alveolitis and
other fibrotic lung diseases; hemolytic anemia;
20 Creutzfeldt-Jakob disease; subacute sclerosing
panencephalitis, Hallerrorden-Spatz disease; and dementia
pugilistica, or any subset thereof;

(E) malignant pathologies involving TNF-secreting
tumors or other malignancies involving TNF, such as, but
25 not limited to, leukemias (acute, chronic myelocytic,
chronic lymphocytic and/or myelodyspastic syndrome);
lymphomas (Hodgkin's and non-Hodgkin's lymphomas, such as
malignant lymphomas (Burkitt's lymphoma or Mycosis
fungoides));

30 (F) cachectic syndromes and other pathologies and
diseases involving excess TNF, such as, but not limited to,
cachexia of cancer, parasitic disease and heart failure;
and

(G) alcohol-induced hepatitis and other forms of
35 chronic hepatitis.

See, e.g., Berkow *et al.*, Eds., *The Merck Manual*, 16th edition, chapter 11, pp. 1380-1529, Merck and Co., Rahway, New Jersey, 1992, incorporated herein by reference.

5   The terms "recurrence", "flare-up" or "relapse" are defined to encompass the reappearance of one or more symptoms of the disease state. For example, in the case of rheumatoid arthritis, a reoccurrence can include the experience of one or more of swollen joints, morning stiffness or joint tenderness.

10   In one embodiment, the invention relates to a method of treating and/or preventing rheumatoid arthritis in an individual comprising co-administering methotrexate and a TNF antagonist to the individual in therapeutically effective amounts.

15   In a second embodiment, the invention relates to a method for treating and/or preventing Crohn's disease in an individual comprising co-administering a methotrexate and a TNF antagonist to the individual in therapeutically effective amounts.

20   In a third embodiment, the invention relates to a method for treating and/or preventing an acute or chronic immune disease associated with an allogenic transplantation in an individual comprising co-administering methotrexate and a TNF antagonist to the individual in therapeutically

25 effective amounts. As used herein, a "transplantation" includes organ, tissue or cell transplantation, such as renal transplantation, cardiac transplantation, bone marrow transplantation, liver transplantation, pancreatic transplantation, small intestine transplantation, skin

30 transplantation and lung transplantation.

     The benefits of combination therapy with methotrexate and TNF antagonists include high clinical response rates for significantly longer durations in comparison with that obtained with treatment with each therapeutic modality

35 separately. In addition, methotrexate significantly

reduces immunogenicity of anti-TNF antibodies, thus permitting administration of multiple dosages of anti-TNF antibodies with enhanced safety. The results described herein suggest that methotrexate can be used to reduce
5 immunogenicity of other antibodies or proteins. Based on the results described herein, methotrexate can be used in other forms of antibody therapy, such as anti-IL-2 antibody therapy. This method is particularly pertinent in therapies other than anti-CD4 antibody therapy.
10 In a further embodiment, the invention relates to compositions comprising methotrexate and a TNF antagonist. The compositions of the present invention are useful for treating a subject having a pathology or condition associated with abnormal levels of a substance reactive
15 with a TNF antagonist, in particular TNF in excess of, or less than, levels present in a normal healthy subject, where such excess or diminished levels occur in a systemic, localized or particular tissue type or location in the body. Such tissue types can include, but are not limited
20 to, blood, lymph, central nervous system (CNS), liver, kidney, spleen, heart muscle or blood vessels, brain or spinal cord white matter or grey matter, cartilage, ligaments, tendons, lung, pancreas, ovary, testes, prostate. Increased or decreased TNF concentrations
25 relative to normal levels can also be localized to specific regions or cells in the body, such as joints, nerve blood vessel junctions, bones, specific tendons or ligaments, or sites of infection, such as bacterial or viral infections.

Tumor Necrosis Factor Antagonists
30 As used herein, a "tumor necrosis factor antagonist" decreases, blocks, inhibits, abrogates or interferes with TNF activity *in vivo*. For example, a suitable TNF antagonist can bind TNF and includes anti-TNF antibodies and receptor molecules which bind specifically to TNF. A

suitable TNF antagonist can also prevent or inhibit TNF
synthesis and/or TNF release and includes compounds such as
thalidomide, tenidap, and phosphodiesterase inhibitors,
such as, but not limited to, pentoxifylline and rolipram.

5   A suitable TNF antagonist that can prevent or inhibit TNF
synthesis and/or TNF release also includes A2b adenosine
receptor enhancers and A2b adenosine receptor agonists
(e.g., 5'-(N-cyclopropyl)-carboxamidoadenosine, 5'-N-
ethylcarboxamidoadenosine, cyclohexyladenosine and R-N^i-

10 phenyl-2-propyladenosine). See, for example, Jacobson
(GB 2 289 218 A), the teachings of which are entirely
incorporated herein by reference. A suitable TNF
antagonist can also prevent or inhibit TNF receptor
signalling.

15 Anti-TNF Antibodies

        As used herein, an "anti-tumor necrosis factor
antibody" decreases, blocks, inhibits, abrogates or
interferes with TNF activity *in vivo*. Anti-TNF antibodies
useful in the methods and compositions of the present

20 invention include monoclonal, chimeric, humanized,
resurfaced and recombinant antibodies and fragments thereof
which are characterized by high affinity binding to TNF and
low toxicity (including human anti-murine antibody (HAMA)
and/or human anti-chimeric antibody (HACA) response). In

25 particular, an antibody where the individual components,
such as the variable region, constant region and framework,
individually and/or collectively possess low immunogenicity
is useful in the present invention. The antibodies which
can be used in the invention are characterized by their

30 ability to treat patients for extended periods with good to
excellent alleviation of symptoms and low toxicity. Low
immunogenicity and/or high affinity, as well as other
undefined properties, may contribute to the therapeutic
results achieved.

An example of a high affinity monoclonal antibody useful in the methods and compositions of the present invention is murine monoclonal antibody (mAb) A2 and antibodies which will competitively inhibit *in vivo* the
5 binding to human TNFα of anti-TNFα murine mAb A2 or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Murine monoclonal antibody A2 and chimeric derivatives thereof, such as cA2, are described in U.S. Application
10 No. 08/192,093 (filed February 4, 1994), U.S. Application No. 08/192,102 (filed February 4, 1994), U.S. Application No. 08/192,861 (filed February 4, 1994), U.S. Application No. 08/324,799 (filed October 18, 1994), and Le, J. et al., International Publication No. WO 92/16553 (published
15 October 1, 1992), which references are entirely incorporated herein by reference. A second example of a high affinity monoclonal antibody useful in the methods and compositions of the present invention is murine mAb 195 and antibodies which will competitively inhibit *in vivo* the
20 binding to human TNFα of anti-TNFα murine 195 or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Other high affinity monoclonal antibodies useful in the methods and compositions of the present invention include
25 murine mAb 114 and murine mAb 199 and antibodies which will competitively inhibit *in vivo* the binding to human TNFα of anti-TNFα murine mAb 114 or mAb 199 or an antibody having substantially the same specific binding characteristics of mAb 114 or mAb 199, as well as fragments and regions
30 thereof. Murine monoclonal antibodies 114, 195 and 199 and the method for producing them are described by Möller, A. et al. (*Cytokine* 2(3):162-169 (1990)), the teachings of which are entirely incorporated herein by reference. Preferred methods for determining mAb specificity and
35 affinity by competitive inhibition can be found in Harlow,

et al., Antibodies: A Laboratory Manual, Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, New York (1988);
Colligan et al., eds., Current Protocols in Immunology,
Greene Publishing Assoc. and Wiley Interscience, New York
5 (1992, 1993); Kozbor et al., Immunol. Today 4:72-79 (1983);
Ausubel et al., eds., Current Protocols in Molecular
Biology, Wiley Interscience, New York (1987, 1992, 1993);
and Muller, Meth. Enzymol. 92:589-601 (1983), which
references are entirely incorporated herein by reference.

10   Additional examples of monoclonal anti-TNF antibodies
that can be used in the present invention are described in
the art (see, e.g., U.S. Application No. 07/943,852 (filed
September 11, 1992); Rathjen et al., International
Publication No. WO 91/02078 (published February 21, 1991);
15 Rubin et al., EPO Patent Publication 0218868 (published
April 22, 1987); Yone et al., EPO Patent Publication
No. 0288088 (October 26, 1988); Liang, et al., Biochem.
Biophys. Res. Comm. 137:847-854 (1986); Meager, et al.,
Hybridoma 6:305-311 (1987); Fendly et al., Hybridoma
20 6:359-369 (1987); Bringman, et al., Hybridoma 6:489-507
(1987); Hirai, et al., J. Immunol. Meth. 96:57-62 (1987);
Moller, et al., Cytokine 2:162-169 (1990), which references
are entirely incorporated herein by reference).

      Chimeric antibodies are immunoglobulin molecules
25 characterized by two or more segments or portions derived
from different animal species. Generally, the variable
region of the chimeric antibody is derived from a non-human
mammalian antibody, such as a murine mAb, and the
immunoglobulin constant region is derived from a human
30 immunoglobulin molecule. Preferably, a variable region
with low immunogenicity is selected and combined with a
human constant region which also has low immunogenicity,
the combination also preferably having low immunogenicity.
"Low" immunogenicity is defined herein as raising
35 significant HACA or HAMA responses in less than about 75%,

or preferably less than about 50% of the patients treated
and/or raising low titres in the patient treated (less than
about 300, preferably less than about 100 measured with a
double antigen enzyme immunoassay) (Elliott et al., Lancet
5   344:1125-1127 (1994), incorporated herein by reference).

As used herein, the term "chimeric antibody" includes
monovalent, divalent or polyvalent immunoglobulins. A
monovalent chimeric antibody is a dimer (HL)) formed by a
chimeric H chain associated through disulfide bridges with
10 a chimeric L chain. A divalent chimeric antibody is a
tetramer (H2L2) formed by two HL dimers associated through
at least one disulfide bridge. A polyvalent chimeric
antibody can also be produced, for example, by employing a
CH region that aggregates (e.g., from an IgM H chain, or μ
15 chain).

Antibodies comprise individual heavy (H) and/or light
(L) immunoglobulin chains. A chimeric H chain comprises an
antigen binding region derived from the H chain of a
non-human antibody specific for TNF, which is linked to at
20 least a portion of a human H chain C region (CH), such as
CH1 or CH2. A chimeric L chain comprises an antigen
binding region derived from the L chain of a non-human
antibody specific for TNF, linked to at least a portion of
a human L chain C region (CL).

25 Chimeric antibodies and methods for their production
have been described in the art (Morrison et al., Proc.
Natl. Acad. Sci. USA 81:6851-6855 (1984); Boulianne et al.,
Nature 312:643-646 (1984); Neuberger et al., Nature
314:268-270 (1985); Taniguchi et al., European Patent
30 Application No. 171496 (published February 19, 1985);
Morrison et al., European Patent Application No. 173494
(published March 5, 1986); Neuberger et al., PCT
Application No. WO 86/01533, (published March 13, 1986);
Kudo et al., European Patent Application No. 184187
35 (published June 11, 1986); Morrison et al., European Patent

Application No. 173494 (published March 5, 1986); Sahagan
et al., *J. Immunol.* 137:1066-1074 (1986); Robinson et al.,
International Publication No. PCT/US86/02269 (published
May 7, 1987); Liu et al., *Proc. Natl. Acad. Sci. USA*
84:3439-3443 (1987); Sun et al., *Proc. Natl. Acad. Sci. USA*
84:214-218 (1987); Better et al., *Science* 240:1041-1043
(1988); and Harlow and Lane, *Antibodies: A Laboratory
Manual*, Cold Spring Harbor Laboratory, New York, 1988).
These references are entirely incorporated herein by
reference.

The anti-TNF chimeric antibody can comprise, for
example, two light chains and two heavy chains, each of the
chains comprising at least part of a human constant region
and at least part of a variable (V) region of non-human
origin having specificity to human TNF, said antibody
binding with high affinity to an inhibiting and/or
neutralizing epitope of human TNF, such as the antibody
cA2. The antibody also includes a fragment or a derivative
of such an antibody, such as one or more portions of the
antibody chain, such as the heavy chain constant or
variable regions, or the light chain constant or variable
regions.

Humanizing and resurfacing the antibody can further
reduce the immunogenicity of the antibody. See, for
example, Winter (U.S. Patent No. 5,225,539 and
EP 239,400 B1), Padlan et al. (EP 519,596 A1) and Pedersen
et al. (EP 592,106 A1). These references are incorporated
herein by reference.

Preferred antibodies useful in the methods and
compositions of the present invention are high affinity
human-murine chimeric anti-TNF antibodies, and fragments or
regions thereof, that have potent inhibiting and/or
neutralizing activity *in vivo* against human TNFα. Such
antibodies and chimeric antibodies can include those
generated by immunization using purified recombinant TNFα

or peptide fragments thereof comprising one or more epitopes.

An example of such a chimeric antibody is cA2 and antibodies which will competitively inhibit *in vivo* the
5 binding to human TNFα of anti-TNFα murine mAb A2, chimeric mAb cA2, or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Chimeric mAb cA2 has been described, for example, in U.S. Application No. 08/192,093 (filed
10 February 4, 1994), U.S. Application No. 08/192,102 (filed February 4, 1994), U.S. Application No. 08/192,861 (filed February 4, 1994), and U.S. Application No. 08/324,799 (filed October 18, 1994), and by Le, J. et al. (International Publication No. WO 92/16553 (published
15 October 1, 1992)); Knight, D.M. et al. (*Mol. Immunol.* 30:1443-1453 (1993)); and Siegel, S.A. et al. (*Cytokine* 7(1):15-25 (1995)). These references are entirely incorporated herein by reference.

Chimeric A2 anti-TNF consists of the antigen binding
20 variable region of the high-affinity neutralizing mouse anti-human TNF IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum
25 half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric A2 is derived from the variable region of the murine A2. Chimeric A2 neutralizes the cytotoxic effect of both natural and recombinant human TNF in a dose dependent
30 manner. From binding assays of cA2 and recombinant human TNF, the affinity constant of cA2 was calculated to be $1.8 \times 10^9 M^{-1}$. Preferred methods for determining mAb specificity and affinity by competitive inhibition can be found in Harlow, et al., *Antibodies: A Laboratory Manual*,
35 Cold Spring Harbor Laboratory Press, Cold Spring Harbor,

New York, 1988; Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992, 1993); Kozbor et al., *Immunol. Today* 4:72-79 (1983); Ausubel et al., eds. *Current*

5   *Protocols in Molecular Biology*, Wiley Interscience, New York (1987, 1992, 1993); and Muller, *Meth. Enzymol.* 92:589-601 (1983), which references are entirely incorporated herein by reference.

        As used herein, the term "antigen binding region"
10 refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antibody region includes the "framework" amino acid residues necessary to maintain the
15 proper conformation of the antigen-binding residues. Generally, the antigen binding region will be of murine origin. In other embodiments, the antigen binding region can be derived from other animal species, such as sheep, rabbit, rat or hamster. Preferred sources for the DNA
20 encoding such a non-human antibody include cell lines which produce antibody, preferably hybrid cell lines commonly known as hybridomas. In one embodiment, a preferred hybridoma is the A2 hybridoma cell line.

        An "antigen" is a molecule or a portion of a molecule
25 capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of selectively binding to an epitope of that antigen. An antigen can have one or more than one epitope.

        The term "epitope" is meant to refer to that portion
30 of the antigen capable of being recognized by and bound by an antibody at one or more of the antibody's antigen binding region. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional
35 structural characteristics as well as specific charge

characteristics. By "inhibiting and/or neutralizing epitope" is intended an epitope, which, when bound by an antibody, results in loss of biological activity of the molecule containing the epitope, *in vivo* or *in vitro*, more
5   preferably *in vivo*, including binding of TNF to a TNF receptor. Epitopes of TNF have been identified within amino acids 1 to about 20, about 56 to about 77, about 108 to about 127 and about 138 to about 149. Preferably, the antibody binds to an epitope comprising at least about 5
10   amino acids of TNF within TNF residues from about 87 to about 107, about 59 to about 80 or a combination thereof. Generally, epitopes include at least about 5 amino acids and less than about 22 amino acids embracing or overlapping one or more of these regions.
15   For example, epitopes of TNF which are recognized by, and/or binds with anti-TNF activity, an antibody, and fragments, and variable regions thereof, include:

    59-80:   Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-
                Cys-Pro-Ser-Thr-His-Val-Leu-Leu-Thr-His-
20   Thr-Ile (SEQ ID NO:1); and/or

    87-108:   Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-Ser-Ala-
                Ile-Lys-Ser-Pro-Cys-Gln-Arg-Glu-Thr-Pro-
                Glu-Gly (SEQ ID NO:2).

    The anti-TNF antibodies, and fragments, and variable
25   regions thereof, that are recognized by, and/or binds with anti-TNF activity, these epitopes block the action of TNFα without binding to the putative receptor binding locus as presented by Eck and Sprang (*J. Biol. Chem. 264(29)*: 17595-17605 (1989) (amino acids 11-13, 37-42, 49-57 and
30   155-157 of hTNFα). Rathjen *et al.*, International Publication No. WO 91/02078 (published February 21, 1991),

incorporated herein by reference, discloses TNF ligands which can bind additional epitopes of TNF.

Antibody Production Using Hybridomas

5   The techniques to raise antibodies to small peptide sequences that recognize and bind to those sequences in the free or conjugated form or when presented as a native sequence in the context of a large protein are well known in the art. Such antibodies can be produced by hybridoma or recombinant techniques known in the art.

10   Murine antibodies which can be used in the preparation of the antibodies useful in the methods and compositions of the present invention have also been described in Rubin et al., EP 0218868 (published April 22, 1987); Yone et al., EP 0288088 (published October 26, 1988); Liang, et al.,

15 *Biochem. Biophys. Res. Comm.* 137:847-854 (1986); Meager, et al., *Hybridoma* 6:305-311 (1987); Fendly et al., *Hybridoma* 6:359-369 (1987); Bringman, et al., *Hybridoma* 6:489-507 (1987); Hirai, et al., *J. Immunol. Meth.* 96:57-62 (1987); Möller, et al., *Cytokine* 2:162-169 (1990).

20   The cell fusions are accomplished by standard procedures well known to those skilled in the field of immunology. Fusion partner cell lines and methods for fusing and selecting hybridomas and screening for mAbs are well known in the art. See, e.g, Ausubel *infra*, Harlow

25 *infra*, and Colligan *infra*, the contents of which references are incorporated entirely herein by reference.

The TNFα-specific murine mAb useful in the methods and compositions of the present invention can be produced in large quantities by injecting hybridoma or transfectoma

30 cells secreting the antibody into the peritoneal cavity of mice and, after appropriate time, harvesting the ascites fluid which contains a high titer of the mAb, and isolating the mAb therefrom. For such *in vivo* production of the mAb with a hybridoma (e.g., rat or human), hybridoma cells are

preferably grown in irradiated or athymic nude mice.
Alternatively, the antibodies can be produced by culturing
hybridoma or transfectoma cells *in vitro* and isolating
secreted mAb from the cell culture medium or recombinantly,
5 in eukaryotic or prokaryotic cells.

In one embodiment, the antibody used in the methods
and compositions of the present invention is a mAb which
binds amino acids of an epitope of TNF recognized by A2,
rA2 or cA2, produced by a hybridoma or by a recombinant
10 host. In another embodiment, the antibody is a chimeric
antibody which recognizes an epitope recognized by A2. In
still another embodiment, the antibody is a chimeric
antibody designated as chimeric A2 (cA2).

As examples of antibodies useful in the methods and
15 compositions of the present invention, murine mAb A2 is
produced by a cell line designated c134A. Chimeric
antibody cA2 is produced by a cell line designated c168A.
c168A was deposited at the American Type Culture
Collection, Rockville, Maryland, as a "Culture Safe
20 Deposit."

"Derivatives" of the antibodies including fragments,
regions or proteins encoded by truncated or modified genes
to yield molecular species functionally resembling the
immunoglobulin fragments are also useful in the methods and
25 compositions of the present invention. The modifications
include, but are not limited to, addition of genetic
sequences coding for cytotoxic proteins such as plant and
bacterial toxins. The fragments and derivatives can be
produced from appropriate cells, as is known in the art.
30 Alternatively, anti-TNF antibodies, fragments and regions
can be bound to cytotoxic proteins or compounds *in vitro*,
to provide cytotoxic anti-TNF antibodies which would
selectively kill cells having TNF on their surface.

"Fragments" of the antibodies include, for example,
35 Fab, Fab', F(ab')$_2$ and Fv. These fragments lack the Fc

fragment of intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)). These fragments are produced from
5 intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

Recombinant Expression of Anti-TNF Antibodies

10 Recombinant and/or chimeric murine-human or human-human antibodies that inhibit TNF can be produced using known techniques based on the teachings provided in U.S. Application No. 08/192,093 (filed February 4, 1994), U.S. Application No. 08/192,102 (filed February 4, 1994),
15 U.S. Application No. 08/192,861 (filed February 4, 1994), U.S. Application No. 08/324,799 (filed on October 18, 1994) and Le, J. et al., International Publication No. WO 92/16553 (published October 1, 1992), which references are entirely incorporated herein by reference. See, e.g.,
20 Ausubel et al., eds. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987, 1992, 1993); and Sambrook et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989), the contents of which are entirely incorporated
25 herein by reference. See also, e.g., Knight, D.M., et al., *Mol. Immunol* 30:1443-1453 (1993); and Siegel, S.A., et al., *Cytokine* 7(1):15-25 (1995), the contents of which are entirely incorporated herein by reference.

The DNA encoding an anti-TNF antibody can be genomic
30 DNA or cDNA which encodes at least one of the heavy chain constant region (Hc), the heavy chain variable region (Hc), the light chain variable region (Lv) and the light chain constant regions (Lc). A convenient alternative to the use of chromosomal gene fragments as the source of DNA encoding

the murine V region antigen-binding segment is the use of cDNA for the construction of chimeric immunoglobulin genes, e.g., as reported by Liu et al. (*Proc. Natl. Acad. Sci.*, *USA 84*:3439 (1987) and *J. Immunology 139*:3521 (1987)),
5 which references are entirely incorporated herein by reference. The use of cDNA requires that gene expression elements appropriate for the host cell be combined with the gene in order to achieve synthesis of the desired protein. The use of cDNA sequences is advantageous over genomic
10 sequences (which contain introns), in that cDNA sequences can be expressed in bacteria or other hosts which lack appropriate RNA splicing systems. An example of such a preparation is set forth below.

   Because the genetic code is degenerate, more than one
15 codon can be used to encode a particular amino acid. Using the genetic code, one or more different oligonucleotides can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual XXX-
20 encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic or prokaryotic cells expressing an anti-TNF antibody or fragment. Such "codon usage rules"
25 are disclosed by Lathe, et al., *J. Mol. Biol. 183*:1-12 (1985). Using the "codon usage rules" of Lathe, a single oligonucleotide, or a set of oligonucleotides, that contains a theoretical "most probable" nucleotide sequence capable of encoding anti-TNF variable or constant region
30 sequences is identified.

   Although occasionally an amino acid sequence can be encoded by only a single oligonucleotide, frequently the amino acid sequence can be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the
35 members of this set contain oligonucleotides which are

capable of encoding the peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is

5 identical to the nucleotide sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner

10 in which one would employ a single oligonucleotide to clone the gene that encodes the protein.

The oligonucleotide, or set of oligonucleotides, containing the theoretical "most probable" sequence capable of encoding an anti-TNF antibody or fragment including a

15 variable or constant region is used to identify the sequence of a complementary oligonucleotide or set of oligonucleotides which is capable of hybridizing to the "most probable" sequence, or set of sequences. An oligonucleotide containing such a complementary sequence

20 can be employed as a probe to identify and isolate the variable or constant region anti-TNF gene (Sambrook et al., infra).

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment

25 of the variable or constant anti-TNF region (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified (using the above-described procedure), synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA

30 preparation derived from cells which are capable of expressing anti-TNF antibodies or variable or constant regions thereof. Single stranded oligonucleotide molecules complementary to the "most probable" variable or constant anti-TNF region peptide coding sequences can be synthesized

35 using procedures which are well known to those of ordinary

skill in the art (Belagaje, et al., J. Biol. Chem.
254:5765-5780 (1979); Maniatis, et al., In: Molecular
Mechanisms in the Control of Gene Expression, Nierlich, et
al., eds., Acad. Press, New York (1976); Wu, et al., Prog.
5 Nucl. Acid Res. Molec. Biol. 21:101-141 (1978); Khorana,
Science 203:614-625 (1979)). Additionally, DNA synthesis
can be achieved through the use of automated synthesizers.
Techniques of nucleic acid hybridization are disclosed by
Sambrook et al., Molecular Cloning: A Laboratory Manual,
10 Cold Spring Harbor Laboratory Press, New York (1989); and
by Haynes, et al., in: Nucleic Acid Hybridization, A
Practical Approach, IRL Press, Washington, DC (1985), which
references are entirely incorporated herein by reference.
Techniques such as, or similar to, those described above
15 have successfully enabled the cloning of genes for human
aldehyde dehydrogenases (Hsu, et al., Proc. Natl. Acad.
Sci. USA 82:3771-3775 (1985)), fibronectin (Suzuki, et al.,
Eur. Mol. Biol. Organ. J. 4:2519-2524 (1985)), the human
estrogen receptor gene (Walter, et al., Proc. Natl. Acad.
20 Sci. USA 82:7889-7893 (1985)), tissue-type plasminogen
activator (Pennica, et al., Nature 301:214-221 (1983)) and
human placental alkaline phosphatase complementary DNA
(Keun, et al., Proc. Natl. Acad. Sci. USA 82:8715-8719
(1985)).

25   In an alternative way of cloning a polynucleotide
encoding an anti-TNF variable or constant region, a library
of expression vectors is prepared by cloning DNA or, more
preferably, cDNA (from a cell capable of expressing an
anti-TNF antibody or variable or constant region) into an
30 expression vector. The library is then screened for
members capable of expressing a protein which competitively
inhibits the binding of an anti-TNF antibody, such as A2 or
cA2, and which has a nucleotide sequence that is capable of
encoding polypeptides that have the same amino acid
35 sequence as anti-TNF antibodies or fragments thereof. In

this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing an anti-TNF antibody or fragment. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to

5　produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment such as in a lambda phage library, expression

10　in prokaryotic cell (e.g., bacteria) or eukaryotic cells, (e.g., mammalian, yeast, insect or, fungus). See, e.g., Ausubel, *infra*, Harlow, *infra*, Colligan, *infra*; Nyyssonen et al. *Bio/Technology* 11:591-595 (1993); Marks et al., *Bio/Technology* 11:1145-1149 (October 1993). Once nucleic

15　acid encoding such variable or constant anti-TNF regions is isolated, the nucleic acid can be appropriately expressed in a host cell, along with other constant or variable heavy or light chain encoding nucleic acid, in order to provide recombinant monoclonal antibodies that bind TNF with

20　inhibitory activity. Such antibodies preferably include a murine or human anti-TNF variable region which contains a framework residue having complementarity determining residues which are responsible for antigen binding.

　　　　Human genes which encode the constant (C) regions of

25　the chimeric antibodies, fragments and regions of the present invention can be derived from a human fetal liver library, by known methods. Human C region genes can be derived from any human cell including those which express and produce human immunoglobulins. The human CH region can

30　be derived from any of the known classes or isotypes of human H chains, including gamma, μ, α, δ or ϵ, and subtypes thereof, such as G1, G2, G3 and G4. Since the H chain isotype is responsible for the various effector functions of an antibody, the choice of CH region will be guided by

35　the desired effector functions, such as complement

fixation, or activity in antibody-dependent cellular cytotoxicity (ADCC). Preferably, the CH region is derived from gamma 1 (IgG1), gamma 3 (IgG3), gamma 4 (IgG4), or μ (IgM). The human CL region can be derived from either

5 human L chain isotype, kappa or lambda.

Genes encoding human immunoglobulin C regions are obtained from human cells by standard cloning techniques (Sambrook, et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor,

10 New York (1989) and Ausubel et al., eds., Current Protocols in Molecular Biology, Wiley Interscience, New York (1987-1993)). Human C region genes are readily available from known clones containing genes representing the two classes of L chains, the five classes of H chains and

15 subclasses thereof. Chimeric antibody fragments, such as F(ab')$_2$ and Fab, can be prepared by designing a chimeric H chain gene which is appropriately truncated. For example, a chimeric gene encoding an H chain portion of an F(ab')$_2$ fragment would include DNA sequences encoding the CH1

20 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

Generally, the murine, human and chimeric antibodies, fragments and regions are produced by cloning DNA segments encoding the H and L chain antigen-binding regions of a

25 TNF-specific antibody, and joining these DNA segments to DNA segments encoding CH and CL regions, respectively, to produce murine, human or chimeric immunoglobulin-encoding genes. Thus, in a preferred embodiment, a fused chimeric gene is created which comprises a first DNA segment that

30 encodes at least the antigen-binding region of non-human origin, such as a functionally rearranged V region with joining (J) segment, linked to a second DNA segment encoding at least a part of a human C region.

Therefore, cDNA encoding the antibody V and C regions and the method of producing a chimeric antibody can involve several steps, outlined below:

1. isolation of messenger RNA (mRNA) from the cell line producing an anti-TNF antibody and from optional additional antibodies supplying heavy and light constant regions; cloning and cDNA production therefrom;

2. preparation of a full length cDNA library from purified mRNA from which the appropriate V and/or C region gene segments of the L and H chain genes can be: (i) identified with appropriate probes, (ii) sequenced, and (iii) made compatible with a C or V gene segment from another antibody for a chimeric antibody;

3. Construction of complete H or L chain coding sequences by linkage of the cloned specific V region gene segments to cloned C region gene, as described above;

4. Expression and production of L and H chains in selected hosts, including prokaryotic and eukaryotic cells to provide murine-murine, human-murine, human-human or human-murine antibodies.

One common feature of all immunoglobulin H and L chain genes and their encoded mRNAs is the J region. H and L chain J regions have different sequences, but a high degree of sequence homology exists (greater than 80%) among each group, especially near the C region. This homology is exploited in this method and consensus sequences of H and L

chain J regions can be used to design oligonucleotides for use as primers for introducing useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments.

5   C region cDNA vectors prepared from human cells can be modified by site-directed mutagenesis to place a restriction site at the analogous position in the human sequence. For example, one can clone the complete human kappa chain C (Ck) region and the complete human gamma-1 C

10 region (C gamma-1). In this case, the alternative method based upon genomic C region clones as the source for C region vectors would not allow these genes to be expressed in bacterial systems where enzymes needed to remove intervening sequences are absent. Cloned V region segments

15 are excised and ligated to L or H chain C region vectors. Alternatively, the human C gamma-1 region can be modified by introducing a termination codon thereby generating a gene sequence which encodes the H chain portion of an Fab molecule. The coding sequences with linked V and C regions

20 are then transferred into appropriate expression vehicles for expression in appropriate hosts, prokaryotic or eukaryotic.

Two coding DNA sequences are said to be "operably linked" if the linkage results in a continuously

25 translatable sequence without alteration or interruption of the triplet reading frame. A DNA coding sequence is operably linked to a gene expression element if the linkage results in the proper function of that gene expression element to result in expression of the coding sequence.

30 Expression vehicles include plasmids or other vectors. Preferred among these are vehicles carrying a functionally complete human CH or CL chain sequence having appropriate restriction sites engineered so that any VH or VL chain sequence with appropriate cohesive ends can be easily

35 inserted therein. Human CH or CL chain sequence-containing

vehicles thus serve as intermediates for the expression of any desired complete H or L chain in any appropriate host.

A chimeric antibody, such as a mouse-human or human-human, will typically be synthesized from genes
5   driven by the chromosomal gene promoters native to the mouse H and L chain V regions used in the constructs; splicing usually occurs between the splice donor site in the mouse J region and the splice acceptor site preceding the human C region and also at the splice regions that
10 occur within the human C, region; polyadenylation and transcription termination occur at native chromosomal sites downstream of the human coding regions.

A nucleic acid sequence encoding at least one anti-TNF antibody fragment may be recombined with vector DNA in
15 accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable
20 joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Ausubel, *supra*, Sambrook, *supra*, entirely incorporated herein by reference, and are well known in the art.

A nucleic acid molecule, such as DNA, is "capable of
25 expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the
30 regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as anti-TNF peptides or antibody fragments in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism
35 to organism and is well known in the analogous art. See,

e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989); and Ausubel, eds., *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987, 1993).

5   Many vector systems are available for the expression of cloned anti-TNF peptide H and L chain genes in mammalian cells (see Glover, ed., *DNA Cloning, Vol. II*, pp. 143-238, IRL Press, Washington, DC, 1985). Different approaches can be followed to obtain complete H2L2 antibodies. It is

10   possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H2L2 antibodies. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both H and

15   L chains can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the

20   resulting cell line with an H chain plasmid containing a second selectable marker. Cell lines producing H2L2 molecules via either route could be transfected with plasmids encoding additional copies of peptides, H, L, or H plus L chains in conjunction with additional selectable

25   markers to generate cell lines with enhanced properties, such as higher production of assembled H2L2 antibody molecules or enhanced stability of the transfected cell lines.

Receptor Molecules

30   Receptor molecules (also referred to herein as soluble TNF receptors) useful in the methods and compositions of the present invention are those that bind TNF with high affinity (see, e.g., Feldmann *et al.*, International Publication No. WO 92/07076 (published April 30, 1992),

incorporated herein by reference) and possess low
immunogenicity. In particular, the 55 kDa (p55 TNF-R) and
the 75 kDa (p75 TNF-R) TNF cell surface receptors are
useful in the present invention. Truncated forms of these
5   receptors, comprising the extracellular domains (ECD) of
the receptors or functional portions thereof, are also
useful in the present invention. Truncated forms of the
TNF receptors, comprising the ECD, have been detected in
urine and serum as 30 kDa and 40 kDa TNF inhibitory binding
10 proteins (Engelmann, H. et al., J. Biol. Chem. 265:1531-
1536 (1990)). TNF receptor multimeric molecules and TNF
immunoreceptor fusion molecules, and derivatives and
fragments or portions thereof, are additional examples of
receptor molecules which are useful in the methods and
15 compositions of the present invention. The receptor
molecules which can be used in the invention are
characterized by their ability to treat patients for
extended periods with good to excellent alleviation of
symptoms and low toxicity. Low immunogenicity and/or high
20 affinity, as well as other undefined properties, may
contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the
present invention comprise all or a functional portion of
the ECD of two or more TNF receptors linked via one or more
25 polypeptide linkers. The multimeric molecules can further
comprise a signal peptide of a secreted protein to direct
expression of the multimeric molecule. These multimeric
molecules and methods for their production have been
described in U.S. Application No. 08/437,533 (filed May 9,
30 1995), the content of which is entirely incorporated herein
by reference.

TNF immunoreceptor fusion molecules useful in the
methods and compositions of the present invention comprise
at least one portion of one or more immunoglobulin
35 molecules and all or a functional portion of one or more

TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor
5 fusion molecule is TNF receptor/IgG fusion protein.

TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer et al., *Eur. J. Immunol.* 21:2883-2886 (1991); Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991);
10 Peppel et al., *J. Exp. Med.* 174:1483-1489 (1991); Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Butler et al., *Cytokine* 6(6):616-623 (1994); Baker et al., *Eur. J. Immunol.* 24:2040-2048 (1994); Beutler et al., U.S. Patent No. 5,447,851; and U.S. Application No. 08/442,133 (filed
15 May 16, 1995)). These references are entirely incorporated herein by reference. Methods for producing immunoreceptor fusion molecules can also be found in Capon et al., U.S. Patent No. 5,116,964; Capon et al., U.S. Patent No. 5,225,538; and Capon et al., *Nature* 337:525-531 (1989),
20 which references are entirely incorporated herein by reference.

Derivatives, fragments, regions and functional portions of the receptor molecules functionally resemble the receptor molecules that can be used in the present
25 invention (i.e., they bind TNF with high affinity and possess low immunogenicity). A functional equivalent or derivative of the receptor molecule refers to the portion of the receptor molecule, or the portion of the receptor molecule sequence which encodes the receptor molecule, that
30 is of sufficient size and sequences to functionally resemble the receptor molecules that can be used in the present invention (i.e., bind TNF with high affinity and possess low immunogenicity). A functional equivalent of the receptor molecule also includes modified receptor
35 molecules that functionally resemble the receptor molecules

that can be used in the present invention (i.e., bind TNF
with high affinity and possess low immunogenicity). For
example, a functional equivalent of the receptor molecule
can contain a "SILENT" codon or one or more amino acid
5 substitutions, deletions or additions (e.g., substitution
of one acidic amino acid for another acidic amino acid; or
substitution of one codon encoding the same or different
hydrophobic amino acid for another codon encoding a
hydrophobic amino acid). See Ausubel et al., Current
10 Protocols in Molecular Biology, Greene Publishing Assoc.
and Wiley-Interscience, New York (1989).

Methotrexate

Presently available oral and intravenous formulations
of methotrexate include Rheumatrex® methotrexate dose pack
15 (Lederle Laboratories, Wayne, NJ); methotrexate tablets
(Mylan Pharmaceuticals Inc., Morgantown, WV; Roxane
Laboratories, Inc., Columbus, OH); and methotrexate sodium
tablets, for injection and injection (Immunex Corporation,
Seattle, WA) and methotrexate LPF® sodium (methotrexate
20 sodium injection) (Immunex Corporation, Seattle, WA).
Methotrexate is also available from Pharmacochemie
(Netherlands). Methotrexate prodrugs, homologs and/or
analogs (e.g., folate antagonists) can also be used in the
methods and compositions of the present invention.
25 Alternatively, other immunosuppressive agents (or drugs
that suppress the immune system) can be used in the methods
and compositions of the present invention.

Administration

TNF antagonists, methotrexate and the compositions of
30 the present invention can be administered to an individual
in a variety of ways. The routes of administration include
intradermal, transdermal (e.g., in slow release polymers),
intramuscular, intraperitoneal, intravenous, subcutaneous,

oral, topical, epidural, buccal, rectal, vaginal and intranasal routes. Any other therapeutically efficacious route of administration can be used, for example, infusion or bolus injection, absorption through epithelial or

5 mucocutaneous linings, or by gene therapy wherein a DNA molecule encoding the therapeutic protein or peptide is administered to the patient, e.g., via a vector, which causes the protein or peptide to be expressed and secreted at therapeutic levels *in vivo*. In addition, the TNF

10 antagonists, methotrexate and compositions of the present invention can be administered together with other components of biologically active agents, such as pharmaceutically acceptable surfactants (e.g., glycerides), excipients (e.g., lactose), carriers, diluents and

15 vehicles. If desired, certain sweetening, flavoring and/or coloring agents can also be added.

The TNF antagonists and methotrexate can be administered prophylactically or therapeutically to an individual. TNF antagonists can be administered prior to,

20 simultaneously with (in the same or different compositions) or sequentially with the administration of methotrexate. For example, TNF antagonists can be administered as adjunctive and/or concomitant therapy to methotrexate therapy.

25 For parenteral (e.g., intravenous, subcutaneous, intramuscular) administration, TNF antagonists, methotrexate and the compositions of the present invention can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically

30 acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain

35 isotonicity (e.g., sodium chloride, mannitol) and chemical

stability (e.g., buffers and preservatives). The
formulation is sterilized by commonly used techniques.

Suitable pharmaceutical carriers are described in
Remington's Pharmaceutical Sciences, A. Osol, a standard
5 reference text in this field of art.

For example, a parenteral composition suitable for
administration by injection is prepared by dissolving 1.5%
by weight of active ingredient in 0.9% sodium chloride
solution.

10 TNF antagonists and methotrexate are administered in
therapeutically effective amounts; the compositions of the
present invention are administered in a therapeutically
effective amount. As used herein, a "therapeutically
effective amount" is such that administration of TNF
15 antagonist and methotrexate, or administration of a
composition of the present invention, results in inhibition
of the biological activity of TNF relative to the
biological activity of TNF when therapeutically effective
amounts of antagonist and methotrexate are not
20 administered, or relative to the biological activity of TNF
when a therapeutically effective amount of the composition
is not administered. A therapeutically effective amount is
preferably an amount of TNF antagonist and methotrexate
necessary to significantly reduce or eliminate signs and
25 symptoms associated with a particular TNF-mediated disease.
As used herein, a therapeutically effective amount is not
necessarily an amount such that administration of the TNF
antagonist alone, or administration of methotrexate alone,
must necessarily result in inhibition of the biological
30 activity of TNF.

Once a therapeutically effective amount has been
administered, a maintenance amount of TNF antagonist alone,
of methotrexate alone, or of a combination of TNF
antagonist and methotrexate can be administered to the
35 individual. A maintenance amount is the amount of TNF

antagonist, methotrexate, or combination of TNF antagonist and methotrexate necessary to maintain the reduction or elimination of the signs and symptoms associated with a particular TNF-mediated disease achieved by the

5 therapeutically effective dose. The maintenance amount can be administered in the form of a single dose, or a series or doses separated by intervals of days or weeks.

The dosage administered to an individual will vary depending upon a variety of factors, including the

10 pharmacodynamic characteristics of the particular antagonists, and its mode and route of administration; size, age, sex, health, body weight and diet of the recipient; nature and extent of symptoms of the disease being treated, kind of concurrent treatment, frequency of

15 treatment, and the effect desired. *In vitro* and *in vivo* methods of determining the inhibition of TNF in an individual are well known to those of skill in the art. Such *in vitro* assays can include a TNF cytotoxicity assay (e.g., the WEHI assay or a radioimmunoassay, ELISA). *In*

20 *vivo* methods can include rodent lethality assays and/or primate pathology model systems (Mathison et al., *J. Clin. Invest.*, 81:1925-1937 (1988); Beutler et al., *Science* 229:869-871 (1985); Tracey et al., *Nature* 330:662-664 (1987); Shimamoto et al., *Immunol. Lett.* 17:311-318 (1988);

25 Silva et al., *J. Infect. Dis.* 162:421-427 (1990); Opal et al., *J. Infect. Dis.* 161:1148-1152 (1990); Hinshaw et al., *Circ. Shock* 30:279-292 (1990)).

TNF antagonist and methotrexate can each be administered in single or multiple doses depending upon

30 factors such as nature and extent of symptoms, kind of concurrent treatment and the effect desired. Thus, other therapeutic regimens or agents (e.g., multiple drug regimens) can be used in combination with the therapeutic co-administration of TNF antagonists and methotrexate. In

35 a particular embodiment, a TNF antagonist is administered

in multiple doses. In another embodiment, methotrexate is administered in the form of a series of low doses separated by intervals of days or weeks. Adjustment and manipulation of established dosage ranges are well within the ability of those skilled in the art.

Usually a daily dosage of active ingredient can be about 0.01 to 100 milligrams per kilogram of body weight. Ordinarily 1 to 40 milligrams per kilogram per day given in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results. Second or subsequent administrations can be administered at a dosage which is the same, less than or greater than the initial or previous dose administered to the individual.

A second or subsequent administration is preferably during or immediately prior to relapse or a flare-up of the disease or symptoms of the disease. For example, second and subsequent administrations can be given between about one day to 30 weeks from the previous administration. Two, three, four or more total administrations can be delivered to the individual, as needed.

Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The present invention will now be illustrated by the following example, which is not intended to be limiting in any way.

EXAMPLES

EXAMPLE 1 Clinical Treatment of Rheumatoid Arthritis By
Multiple Infusions of an Anti-TNF Antibody With
and Without Methotrexate

5   A randomized, double-blind, placebo controlled study
was conducted to evaluate the safety and efficacy of a
chimeric monoclonal anti-TNF antibody (cA2) following
multiple infusions of 1, 3 or 10 mg/kg cA2, alone or in
combination with methotrexate, compared to multiple
10   infusions of placebo in combination with methotrexate, in
the treatment of rheumatoid arthritis (RA) in patients.

Patients

One hundred one (101) patients at six European centers
who had been using methotrexate for at least 6 months, had
15   been on a stable dose of 7.5 mg/wk for at least 4 weeks,
and had active disease (according to the criteria of the
American College of Rheumatology) with erosive changes on
X-rays of hands and feet, were enrolled in the trial.
Active disease was defined by the presence of six or more
20   swollen joints plus at least three of four secondary
criteria (duration of morning stiffness ≥45 minutes; ≥6
tender or painful joints; erythrocyte sedimentation rate
(ESR) ≥28 mm/hour; C-reactive protein (CRP) ≥20 mg/l.
In patients using corticosteroids (≤ 7.5 mg/day) or
25   non-steroidal anti-inflammatory drugs (NSAIDs), the doses
had been stable for 4 weeks prior to screening. The dose
of corticosteroids remained stable throughout trial
participation. The dose of NSAID typically also remained
stable throughout trial participation.

Study Infusions

The chimeric monoclonal anti-TNF antibody (cA2) was
supplied as a sterile solution containing 5 mg cA2 per ml
of 0.01 M phosphate-buffered saline in 0.15 M sodium
5 chloride with 0.01% polysorbate 80, pH 7.2. The placebo
vials contained 0.1% human serum albumin in the same
buffer. Before use, the appropriate amount of cA2 or
placebo was diluted to 300 ml in sterile saline by the
pharmacist, and administered intravenously via a 0.2 μm in-
10 line filter over 2 hours. The characteristics of the
placebo and cA2 infusion bags were identical, and the
investigators and patients did not know which infusion was
being administered.

Assessments

15 Patients were randomized to one of seven treatment
groups. The number of patients in each dose (or treatment)
group is indicated in Table 1. Each of the 101 patients
received multiple infusions of either 0, 1, 3 or 10 mg/kg
cA2. Infusions were to be administered at weeks 0, 2, 6,
20 10 and 14. Starting at week 0, the patients were receiving
7.5 mg/wk of methotrexate (Pharmacochemie, Netherlands) or
3 placebo tablets/week (Pharmacochemie, Netherlands).
Patients were monitored for adverse events during infusions
and regularly thereafter, by interviews, physical
25 examination, and laboratory testing.

The six primary disease-activity assessments were
chosen to allow analysis of the response in individual
patients according to the Paulus index (Paulus, et al.,
Arthritis Rheumatism 33:477-484 (1990), the teachings of
30 which are incorporated herein by reference). The
assessments contributing to this index were the tender
joint and swollen joint scores (60 and 58 joints,
respectively, hips not assessed for swelling; graded 0-3),
the duration of morning stiffness (minutes), the patient's

and physician's assessment of disease severity (on a
5-point scale, ranging from 1 (symptom-free) to 5 (very
severe), and erythrocyte sedimentation rate (ESR).
Patients were considered to have responded if at least four
5   of the six variables improved, defined as at least 20%
improvement in the continuous variables, and at least two
grades of improvement or improvement from grade 2 to 1 in
the two disease-severity assessments (Paulus 20% response).
Improvements of at least 50% in the continuous variables
10 were also used (Paulus 50% response).

      Other disease-activity assessments included the pain
score (0-10 cm on a visual analogue scale (VAS)), an
assessment of fatigue (0-10 cm VAS), and grip strength
(0-300 mm Hg, mean of three measurements per hand by
15 sphygmomanometer cuff).

      The ESR was measured at each study site with a
standard method (Westergen). C-reactive protein (CRP) was
measured by rate nephelometry (Abbott fluorescent
polarizing immunoassay). See also, Elliott et al., Lancet
20 344:1105-1110 (1994); Elliott et al., Lancet 344:1125-1127
(1994); and Elliott et al., Arthritis Rheum. 36(12):1681-
1690 (1993), which references are entirely incorporated
herein by reference.

      Evaluations were performed at weeks 1, 2, 4, 6, 8, 10,
25 12, 14, 16, 18, 20, 22 and 26.

Results

      The 101 patients were randomized to one of seven
treatment (or dose) groups. The patients enrolled in each
dose group were well matched for baseline demographics.
30 Disease duration and swollen and tender joint counts at
baseline were also well-balanced across the groups
(Table 1). Table 1 also shows the maximum methotrexate
dose administered within 6 months prior to randomization.
Median maximum doses for each group ranged between 10 and

15 mg/week; there were no significant differences amongst
the treatment groups (p=0.404).

TABLE 1   Baseline Disease Characteristics Joint Counts

| | Placebo | 1 mg/kg cA2 | |
	MTX+	MTX+	MTX-
Disease dur. (yrs)			
Pts evaluated	14	14	15
Mean±SD	7.6±4.0	14.3±12.1	7.6±6.0
Median	6.9	11.4	5.2
IQ range	(4.3, 11.5)	(3.3, 24.7)	(3.4, 9.0)
Range	(1.8, 14.2)	(0.7, 37.3)	(2.5, 21.3)
Number of Swollen joints, Paulus joint set (0-58)			
Pts evaluated	14	14	15
Mean±SD	18.1±8.6	16.9±7.8	21.2±11.2
Median	16.5	15.5	20.0
IQ range	(12.0, 25.0)	(10.0, 25.0)	(10.0, 33.0)
Range	(6.0, 38.0)	(6.0, 29.0)	(7.0, 40.0)
Number of tender joints, Paulus joint set (0-60)			
Pts evaluated	14	14	15
Mean±SD	31.5±14.2	19.1±10.7	29.9±17.1
Median	27.0	16.0	30.0
IQ range	(22.0, 44.0)	(13.0, 30.0)	(14.0, 45.0)
Range	(8.0, 52.0)	(2.0, 39.0)	(6.0, 58.0)
Max dose MTX prev. 6 mo (mg/kg)			
Pts evaluated	14	14	15
Mean±SD	13.8±3.9	11.6±3.5	12.8±5.6
Median	15.0	11.3	12.5
IQ range	(10.0, 15.0)	(10.0, 12.5)	(10.0, 15.0)
Range	(7.5, 20.0)	(7.5, 20.0)	(7.5, 30.0)

MTX = Methotrexate

TABLE 1   Continued

| | Treatment Groups | |
| | 3 mg/kg cA2 | |
	MTX+	MTX-
Disease dur. (yrs)		
Pts evaluated Mean±SD Median IQ range Range	15 12.1±9.0 11.9 (4.3, 16.4) (0.7, 30.5)	14 7.8±4.3 7.7 (4.6, 9.8) (1.4, 17.4)
Number of Swollen joints, Paulus joint set (0-58)		
Pts evaluated Mean±SD Median IQ range Range	15 17.7±5.9 16.0 (13.0, 22.0) (10.0, 29.0)	14 19.7±9.9 17.0 (11.0, 32.0) (8.0, 34.0)
Number of tender joints, Paulus joint set (0-60)		
Pts evaluated Mean±SD Median IQ range Range	15 24.5±14.4 21.0 (12.0, 32.0) (10.0, 52.0)	14 31.2±11.7 31.0 (23.0, 39.0) (9.0, 52.0)
Max dose MTX prev. 6 mo (mg/kg)		
Pts evaluated Mean±SD Median IQ range Range	14 11.6±3.3 10.0 (10.0, 15.0) (7.5, 17.5)	13 11.7±4.8 10.0 (7.5, 12.5) (7.5, 25.0)

MTX = Methotrexate

TABLE 1 Continued

Treatment Groups

| | 10 mg/kg cA2 | | All Patients | Treatment effect p-value |
	MTX+	MTX-		
Disease dur. (yrs)				
Pts evaluated Mean±SD Median IQ Range Range	14 11.1±7.4 10.7 (4.5, 15.5) (1.4, 24.1)	15 9.7±7.4 7.6 (4.9, 14.9) (1.1, 24.3)	101 10.0±7.8 7.6 (4.3, 14.4) (0.7, 37.3)	0.634
Number of swollen joints, Paulus joint set (0-58)				
Pts evaluated Mean±SD Median IQ Range Range	14 21.1±8.2 19.5 (15.0, 31.0) (10.0, 34.0)	15 17.8±8.7 17.0 (11.0, 21.0) (7.0, 41.0)	101 18.9±8.7 18.0 (12.0, 25.0) (6.0, 41.0)	0.643
Number of tender joints, Paulus joint set (0-60)				
Pts evaluated Mean±SD Median IQ Range Range	14 26.5±12.0 25.5 (21.0, 38.0) (8.0, 44.0)	15 26.2±11.7 23.0 (17.0, 35.0) (11.0, 48.0)	101 27.0±13.5 25.0 (15.0, 38.0) (2.0, 58.0)	0.135
Max dose MTX prev. 6 mo (mg/kg)				
Pts evaluated Mean±SD Median IQ Range Range	14 12.7±5.0 10.0 (10.0, 15.0) (7.5, 25.0)	15 12.5±3.0 12.5 (10.0, 15.0) (7.5, 20.0)	99 12.4±4.2 12.5 (10.0, 15.0) (7.5, 30.0)	0.404

MTX = Methotrexate

The pre-specified primary analysis in this trial was
the comparison of the total time of clinical response
during the 26-week follow-up period. The results for the
primary analysis are shown in Table 2. The duration of
response of all cA2-treated groups, with the exception of
the 1 mg/kg group not receiving methotrexate, was
significantly improved (p<0.001) compared to the placebo
group receiving methotrexate alone.
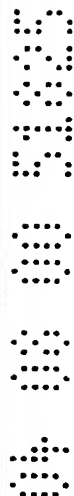
TABLE 2   Total Time of Response* Based On Paulus 20% Criteria

Treatment Groups

Total time of response in weeks	Placebo MTX+ (n=14)	1 mg/kg cA2		3 mg/kg cA2		10 mg/kg cA2		Treatment effect p-value
		MTX+ (n=14)	MTX- (n=15)	MTX+ (n=15)	MTX- (n=14)	MTX+ (n=13)	MTX- (n=15)	
Median	0	16.6	2.6	16.5	17.2	>23.1	10.4	<0.001
Minimum	0	0	0	0	0	0	0	
25th percentile	0.0	6.2	2.0	7.0	4.0	2.6	6.9	
75th percentile	0.0	22.5	8.0	>20.1	20.7	>24.6	>23.1	
Maximum	>15.1	>26.9	15.1	>24.9	>25.9	>25.6	>26.4	
p-val vs. MTX alone		<0.001	0.119	<0.001	<0.001	<0.001	<0.001	

*Patients were followed through 26 weeks following the initial infusion of cA2

-47-

The response rates at Paulus 20% are shown in Table 3. Drop-outs were considered as non-responders subsequent to their dropping out from the study. With the exception of the 1 mg/kg group not receiving methotrexate, all of the
5 cA2-treated groups demonstrated clinical benefit through 14 weeks when the last dose of cA2 was received. Sustained clinical benefit was observed through 26 weeks (the last follow-up visit) in patients who received 3 or 10 mg/kg cA2 with methotrexate. Approximately one-half of the patients
10 who received 3 mg/kg cA2 with methotrexate demonstrated continued clinical benefit at 26 weeks.

TABLE 3   Number of Patients Responding According To Paulus 20% Criteria At Each Evaluation Visit

	Treatment Groups		
	Placebo	1 mg/kg cA2	
	MTX+ (n=14)	MTX+ (n=14)	MTX- (n=15)
Pts with any response	21% (3/14)	93% 13/14	80% 12/15
p-value vs MTX alone		<0.001	0.006
Time post-infusion			
1 Week	0% (0/14)	31% (4/13)	53% (8/15)
2 Weeks	7% (1/14)	64% (9/14)	57% (8/14)
4 Weeks*	0% (0/14)	79% 11/14	33% (5/15)
6 Weeks	0% (0/14)	71% 10/14	27% (4/15)
8 Weeks*	14% (2/14)	64% (9/14)	20% (3/15)
10 Weeks	7% (1/14)	71% 10/14	20% (3/15)
12 Weeks*	7% (1/14)	57% (8/14)	13% (2/15)
14 Weeks	0% (0/14)	71% 10/14	7% (1/15)
16 Weeks*	14% (2/14)	64% (9/14)	7% (1/15)
18 Weeks	21% (3/14)	50% (7/14)	13% (2/15)
20 Weeks	7% (1/14)	54% (7/13)	13% (2/15)
22 Weeks	7% (1/14)	46% (6/13)	0% (0/15)
26 Weeks*	7% (1/14)	21% (3/14)	7% (1/15)

*Evaluation visits pre-specified for analysis.

Table 3 Continued

Treatment Groups

	3 mg/kg cA2		10 mg/kg cA2		
	MTX+ (n=15)	MTX- (n=14)	MTX+ (n=13)	MTX- (n=15)	Treatment effect p-value
Pts with any response	80% 12/15	79% 11/14	85% 11/13	80% 12/15	<0.001
p-value vs MTX alone	0.002	0.002	0.001	0.004	
Time post-infusion					
1 Week	27% (4/15)	43% (6/14)	31% (4/13)	60% (9/15)	
2 Weeks	27% (4/15)	43% (6/14)	62% (8/13)	53% (8/15)	
4 Weeks*	40% (6/15)	64% (9/14)	54% (7/13)	53% (8/15)	0.002
6 Weeks	47% (7/15)	50% (7/14)	54% (7/13)	47% (7/15)	
8 Weeks*	60% (9/15)	71% 10/14	69% (9/13)	40% (6/15)	0.003
10 Weeks	67% 10/15	64% (9/14)	69% (9/13)	53% (8/15)	
12 Weeks*	67% 10/15	64% (9/14)	62% (8/13)	60% (8/13)	<0.001
14 Weeks	60% (9/15)	57% (8/14)	77% 10/13	53% (8/15)	
16 Weeks*	67% 10/15	64% (9/14)	54% (7/13)	67% 10/15	<0.001
18 Weeks	71% 10/14	69% (9/13)	62% (8/13)	57% (8/14)	
20 Weeks	53% (8/15)	43% (6/14)	54% (7/13)	53% (8/15)	
22 Weeks	47% (7/15)	36% (5/14)	54% (7/13)	33% (5/15)	
26 Weeks*	47% (7/15)	21% (3/14)	54% (7/13)	33% (5/15)	0.013

* Evaluation visits pre-specified for analysis.

The response rates at Paulus 50% are shown in Table 4. The magnitude of the clinical benefit of cA2 treatment was substantial. The majority of patients were responding to cA2 treatment according to the 50% Paulus criteria.

TABLE 4 Number of Patients Responding According To Paulus 50% Criteria At Each Evaluation Visit

	Placebo	1 mg/kg cA2	
		Treatment Groups	
	MTX+ (n=14)	MTX+ (n=14)	MTX- (n=15)
Pts with any response	14.3% (2/14)	85.7% (12/14)	40.0% (6/15)
p-value vs MTX alone		<0.001	0.079
Time post-infusion			
1 Week	0.0% (0/14)	7.7% (1/13)	26.7% (4/15)
2 Weeks	0.0% (0/14)	21.4% (3/14)	28.6% (4/14)
4 Weeks*	0.0% (0/14)	57.1% (8/14)	13.3% (2/15)
6 Weeks	0.0% (0/14)	57.1% (8/14)	0.0% (0/15)
8 Weeks*	7.1% (1/14)	50.0% (7/14)	0.0% (0/15)
10 Weeks	0.0% (0/14)	57.1% (8/14)	0.0% (0/15)
12 Weeks*	7.1% (1/14)	50.0% (7/14)	6.7% (1/15)
14 Weeks	0.0% (0/14)	57.1% (8/14)	6.7% (1/15)
16 Weeks*	0.0% (0/14)	64.3% (9/14)	6.7% (1/15)
18 Weeks	7.1% (1/14)	50.0% (7/14)	6.7% (1/15)
20 Weeks	7.1% (1/14)	53.8% (7/13)	0.0% (0/15)
22 Weeks	0.0% (0/14)	38.5% (5/13)	0.0% (0/15)
26 Weeks*	0.0% (0/14)	21.4% (3/14)	6.7% (1/15)

*Evaluation visits pre-specified for analysis.

TABLE 4 Continued

Treatment Groups

	3 mg/kg cA2		10 mg/kg cA2		
	MTX+ (n=15)	MTX- (n=14)	MTX+ (n=13)	MTX- (n=15)	Treatment effect p-value
Pts with any response	73.3% (11/15)	64.3% (9/14)	76.9% (10/13)	66.7% (10/15)	<0.001
p-value vs MTX alone	0.001	0.008	0.002	0.009	
Time post-infusion					
1 Week	0.0% (0/15)	35.7% (5/14)	7.7% (1/13)	26.7% (4/15)	
2 Weeks	6.7% (1/15)	28.6% (4/14)	15.4% (2/13)	20.0% (3/15)	
4 Weeks*	13.3% (2/15)	28.6% (4/14)	46.2% (6/13)	40.0% (6/15)	0.006
6 Weeks	26.7% (4/15)	42.9% (6/14)	38.5% (5/13)	33.3% (5/15)	
8 Weeks*	40.0% (6/15)	50.0% (7/14)	69.2% (9/13)	33.3% (5/15)	<0.001
10 Weeks	40.0% (6/15)	50.0% (7/14)	69.2% (9/13)	40.0% (6/15)	
12 Weeks*	60.0% (9/15)	35.7% (5/14)	61.5% (8/13)	40.0% (6/15)	<0.001
14 Weeks	40.0% (6/15)	35.7% (5/14)	61.5% (8/13)	40.0% (6/15)	
16 Weeks*	60.0% (9/15)	50.0% (7/14)	53.8% (7/13)	40.0% (6/15)	<0.001
18 Weeks	71.4% (10/14)	46.2% (6/13)	61.5% (8/13)	57.1% (8/14)	
20 Weeks	53.3% (8/15)	35.7% (5/14)	46.2% (6/13)	40.0% (6/15)	
22 Weeks	46.7% (7/15)	14.3% (2/14)	53.8% (7/13)	26.7% (4/15)	
26 Weeks*	40.0% (6/15)	14.3% (2/14)	46.2% (6/13)	20.0% (3/15)	0.008

*Evaluation visits pre-specified for analysis.

Commensurate with the clinical response rates shown in
Tables 2-4, most of the patients in the treatment groups
demonstrating effectiveness of cA2 treatment received all 5
infusions of cA2 (Table 5). The principle reason for
5 patients not receiving the complete dose regimen was
because of lack of efficacy in the placebo group
(methotrexate alone) and in the 1 mg/kg group not receiving
methotrexate. All 15 patients in the 3 mg/kg group that
received methotrexate completed the 5-infusion dose
10 regimen.

TABLE 5   Number of Infusions Completed

	Treatment Groups							
	Placebo	1 mg/kg cA2		3 mg/kg cA2		10 mg/kg cA2		Treatment effect p-value
	MTX+ (n=14)	MTX+ (n=14)	MTX- (n=15)	MTX+ (N=15)	MTX- (n=14)	MTX+ (n=14)	MTX- (n=15)	
Pts with complete[a] infusions								
5 infusions	6 (42.86%)	12 (85.71%)	8 (53.33%)	15 (100.00%)	12 (85.71%)	12 (85.71%)	12 (80.00%)	0.003
4 infusions	0 (0.00%)	1 (7.14%)	0 (0.00%)	0 (0.00%)	1 (7.14%)	1 (7.14%)	0 (0.00%)	
3 infusions	2 (14.29%)	1 (7.14%)	6 (40.00%)	0 (0.00%)	0 (0.00%)	1 (7.14%)	1 (6.67%)	
2 infusions	5 (35.71%)	0 (0.00%)	1 (6.67%)	0 (0.00%)	1 (7.14%)	0 (0.00%)	2 (13.33%)	
1 infusion	1 (7.14%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	

[a]Patients are counted only once for the first group for which they qualify (5 infusions> 4 infusions etc....). Patients were only counted if they had completed the entire infusion.

Results for measures of swollen and tender joint counts and the physician and patient global assessments are shown in Figures 1-4. The median results in Figures 1-4 were reported for each evaluation visit based only on the
5   patients with data collected. That is, a last observation carried forward approach was not used for patients who dropped out. Instead, the number of patients with data that comprise each point on the graph was reported at the bottom of the figures.
10   Despite the number of drop-outs in the placebo group and the 1 mg/kg group not receiving methotrexate, the results in Figures 1-4 demonstrate that cA2 treatment in combination with methotrexate profoundly reduces disease activity for all of the traditional measurements of disease
15   activity, approaching near remission in many patients.
        Results for a commonly used serum marker of inflammatory activity, C-reactive protein (CRP) are shown in Figure 5. Treatment with cA2 produced a rapid decrease in CRP concentration which was sustained through 26 weeks
20   in the patients who received 3 or 10 mg/kg cA2.
        Results for the Health Assessment Questionnaire (HAQ) are shown in Figure 6. This measurement of quality of life/disability demonstrated improvement over time corresponding with the clinical improvement observed in
25   patients treated with cA2. In the patients treated with 3 mg/kg cA2 and methotrexate, the HAQ decreased from 2.0 at baseline to 1.1 at 22 weeks.

Pharmacokinetics of cA2

        Serum concentrations of cA2 were obtained in all
30   patients in this study. The serum concentration in each patient plotted over time according to the cA2 dose group is shown in Figure 7. Data plotted are the serum cA2 concentrations obtained just before the administration of cA2 at weeks 2, 6, 10 and 14 and then at weeks 18 and 26.

These sampling times were selected to best demonstrate the stability of the cA2 concentration during the multiple dose regimen and the decline in serum cA2 concentration after the last dose was administered. For purposes of data presentation, the scales for cA2 concentration for each graph are condensed as the cA2 dose was increased.

Substantial differences were observed for the cA2 serum concentration over time in the 1 mg/kg dose groups according to whether patients received methotrexate. Most of the patients receiving 1 mg/kg cA2 with methotrexate demonstrated measurable cA2 concentrations through 18 weeks, although it appeared that there was a tendency for the concentration to decline over time. In sharp contrast, the majority of patients who received 1 mg/kg cA2 without methotrexate were not able to maintain measurable serum concentrations of cA2 over time. As discussed herein, the inability to maintain serum cA2 in these patients was associated with a high rate of neutralizing antibody formation.

In contrast to the 1 mg/kg groups, patients who received either 3 mg/kg cA2 or 10 mg/kg cA2 were able to maintain serum cA2 concentrations through the multiple dose regimen. However, even in those dose groups, there was evidence that concomitant treatment with methotrexate was associated with high cA2 serum concentrations. As shown in Figure 8, the median serum cA2 concentration in both the 3 and 10 mg/kg dose groups receiving methotrexate was higher than in the corresponding groups not receiving methotrexate.

Immune Responses to cA2

Serum samples were collected through 26 weeks from all patients and analyzed for human anti-chimeric antibodies (HACA) to cA2. The results for HACA responses for each cA2 treatment group are shown in Table 6. It should be noted

that in several patients in the 3 mg/kg group and in most
patients in the 10 mg/kg group, cA2 was still present in
the 26-week sample and could potentially interfere with the
detection of HACA in the assay. However, it could also be
5   reasoned that if neutralizing antibodies were present at 26
weeks, then cA2 should not be present. Therefore, in
presenting the data in Table 6, results for the immune
response rate are shown not including patients with serum
cA2 at 26 weeks and including patients with serum cA2 at 26
10 weeks, assuming that if cA2 was present at 26 weeks, the
patient did not have a positive HACA response.
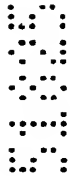
TABLE 6   HACA Responses

	1 mg/kg		3 mg/kg		10 mg/kg	
	MTX+	MTX-	MTX+	MTX-	MTX+	MTX-
HACA responses not including pts with 26-week serum cA2	2/13 (15.4%)	8/15 (53.3%)	0/10 (0%)	3/12 (25.0%)	0/2 (0%)	1/10 (10%)
HACA responses including pts with 26-week serum cA2[1]	2/13 (15.4%)	8/15 (53.3%)	0/15 (0%)	3/14 (21.4%)	0/14 (0%)	1/15 (6.7%)

[1] Patients with a measurable 26-week serum cA2 concentration were considered negative for a HACA response for this analysis.

The results in Table 6 demonstrate that concomitant methotrexate treatment suppresses the immune response to cA2, enabling stable pharmacokinetics to be achieved in a multiple dose regimen of cA2. This effect was also found

5 after combined anti-CD4/anti-TNF antibody treatment in mice with collagen-induced arthritis and described in U.S. Application No. 08/607,419, filed February 28, 1996, the teachings of which are entirely incorporated herein by reference.

10 Clinical Safety

Two out of 86 patients (with most patients receiving 5 treatments) experienced multisystem infusion-related reactions with retreatment. Multisystem, infusion-related reactions include headache, fever, facial flushing,

15 pruritus, myalgia, nausea, chest tightness, dyspnea, vomiting, erythema, abdominal discomfort, diaphoresis, shivers, hypertension, lightheadedness, hypotension, palpitations and somnolence.

Hypersensitivity reactions, as described herein, may

20 occur whenever protein-containing materials, such as cA2, are administered. Thus, it is unclear whether these symptoms represent an immunologic event or physical factors such as infusion rate and immunoglobulin aggregation. Investigators have reported that symptoms resolve in some

25 patients by decreasing the rate of the infusion. Previous literature reports indicate that vasomotor symptoms have been observed in patients receiving intravenous immunoglobulin therapy (Berkman et al., Ann. Intern Med. 112:278-292 (1990); Ochs et al., Lancet 2:1158-1159

30 (1980)).

One patient developed hypotension during all three infusions of 10 mg/kg cA2. The patient did not display clinical signs of hypotension and did not require medical

treatment, but, in keeping with predefined safety criteria, the treatment schedule of this patient was discontinued.

One patient treated with 3 infusions of 10 mg/kg of cA2 and with 7.5 mg/week methotrexate developed symptoms of

5 sepsis as a result of staphylococcal pneumonia 2 weeks after her last study visit and 14 weeks after her last infusion with cA2. Six days after developing symptoms she was admitted to the hospital and treated. She died one day later. (This patient had not proceeded with the fourth

10 infusion for reasons unrelated to the sepsis.) Patients with RA who develop infections have a worse than expected outcome. Wolfe and coworkers have reported an observed:expected ratio for death due to pneumonia of 5.307 and an observed:expected ratio for death due to infections

15 (excluding pneumonia) of 6.213 in RA patients from the ARAMIS database (Wolfe et al., Arthritis Rheumatism 4:481-494 (1994)).

One patient experienced a serious postoperative infection following cataract surgery 9 weeks after the

20 fifth and last infusion of 3 mg/kg of cA2 (with 7.5 mg/week methotrexate), leading to removal of the eye. This patient was receiving prednisolone (7 mg/day). The incidence of endophthalmitis after cataract extraction has been reported to be between 0.072 and 0.093% (Kattan et al.,

25 Ophthalmology 98(9):1147-1148 (1991)) and may be heightened in patients receiving corticosteroid therapy.

Eight (9%) of 87 patients developed double stranded (ds)-DNA antibodies following multiple infusions of cA2. Measurements were performed at baseline, week 8, 16 and 26

30 (12 weeks following the last infusion). In these patients with antibodies against ds-DNA, there was a trend toward a lower level in antibodies at the last evaluation, with two patients being negative.

One patient developed dyspnea, pleuritic chest pain

35 and a rebound of arthritis activity at study week 14 (four

weeks after the fourth infusion of 3 mg/kg of cA2).
Symptoms resolved and she received her fifth dose of cA2.
Symptoms recurred 3 weeks later. Examination of the serial
blood samples revealed that the test for antinuclear

5 antibodies and anti ds-DNA antibodies were negative prior
to treatment, but became positive at week 6 of the study.
The patient's symptoms responded to oral prednisolone
20-30 mg daily. The working diagnosis was systemic lupus
erythematosus (SLE). The patient currently does not have

10 symptoms of SLE but has active RA.

     To date, although antibodies to ds-DNA have been
detected in patients treated with cA2, they generally
represent transient increases and only one patient has been
symptomatic. In patients who have had sufficient

15 follow-up, anti-ds-DNA antibodies have resolved with
discontinuation of treatment.

     In summary, treatment with cA2 is well tolerated. The
reductions in disease activity produced by cA2 are
significant as supported by the findings of a low placebo

20 response rate. High clinical response rates are obtained
with a multiple dose regimen of 3 mg/kg cA2 in combination
with 7.5 mg/wk methotrexate and can be sustained through 26
weeks. This dose regimen is considered preferable to the
1 mg/kg plus methotrexate regimen because better

25 pharmacokinetics are obtained, virtually no immune response
was detected and the clinical response is better sustained
following the last treatment with cA2. The clinical
benefit obtained by increasing the dose regimen to 10 mg/kg
cA2 plus methotrexate is similar to that observed with the

30 3 mg/kg cA2 plus methotrexate regimen.

     Thus, the results of this study indicate that
treatment with a multiple dose regimen of cA2 as adjunctive
and/or concomitant therapy to methotrexate therapy, in RA
patients whose disease is incompletely controlled by

35 methotrexate, produces a highly beneficial or synergistic

clinical response that can be sustained through 26 weeks. The benefit produced by cA2 generally exceeds 50% reductions in the traditional measurements of rheumatoid arthritis (swollen and tender joints, patient and physician

5 global disease assessments) and achieves near clinical remission in many patients. Accordingly, the results of this study indicate that treatment with multiple infusions of cA2 as adjunctive and/or concomitant therapy to methotrexate therapy is an important and efficacious

10 therapeutic approach for treating RA in patients.

EXAMPLE 2 <u>Clinical Treatment of Rheumatoid Arthritis By Single Infusion of an Anti-TNF Antibody In Patients Receiving Methotrexate</u>

A randomized, double-blind, placebo controlled study

15 was conducted to evaluate the effects of a single infusion of placebo, 5, 10 or 20 mg/kg cA2 in combination with methotrexate, administered at a dose of 10 mg/week, in the treatment of rheumatoid arthritis (RA) in patients.

<u>Patients</u>

20 Twenty-eight (28) RA patients at three centers in the United States who, despite receiving three months therapy with methotrexate administered at a stable dose of 10 mg/wk for at least 4 weeks prior to screening, still had active disease according to the criteria of the American College

25 of Rheumatology, were enrolled in the study. Active disease was defined by the presence of six or more swollen joints plus at least three of four secondary criteria (duration of morning stiffness ≥ 45 minutes; ≥ 6 tender or painful joints; erythrocyte sedimentation rate (ESR)

30 ≥ 28 mm/hour; C-reactive protein (CRP) ≥ 20 mg/l.

Patients taking NSAIDs and corticosteroids (prednisone) at screening were allowed to continue at stable doses (7.5 mg/day).

Study Infusions

The chimeric monoclonal anti-TNF antibody (cA2) was supplied as a sterile solution containing 5 mg cA2 per ml of 0.01 M phosphate-buffered saline in 0.15 M sodium
5 chloride with 0.01% polysorbate 80, pH 7.2. The placebo vials contained 0.1% human serum albumin in the same buffer. Before use, the appropriate amount of cA2 or placebo was diluted to 300 ml in sterile saline by the pharmacist, and administered intravenously via a 0.2 μm
10 in-line filter over 2 hours. The characteristics of the placebo and cA2 infusion bags were identical, and the investigators and patients did not know which infusion was being administered.

Assessments

15   Patients were randomized to one of four treatment groups (7 patients per group). Each of the 28 patients received a single dose of either 0, 5, 10 or 20 mg/kg cA2 and were followed for 12 weeks. Patients continued treatment with methotrexate (Pharmacochemie, Netherlands)
20 administered at 10 mg/week throughout the study. Patients were monitored for adverse events during infusions and regularly thereafter, by interviews, physical examination, and laboratory testing.

     The primary measurement of clinical response was
25 defined by the ACR preliminary definition of response (Felson et al., Arthritis Rheumatism 38(6):727-735 (1995)). Patients were considered to have a response if they had a 20% reduction in swollen and tender joint count, and had experienced a 20% reduction in 3 of the 5 following
30 assessments: patient's assessment of pain (VAS), patient's global assessment of disease activity (VAS), physician's global assessment of disease activity (VAS), patient's assessment of physical function (HAQ), and an acute phase

reactant (ESR). The ESR was measured at each study site with a standard method (Westergen).

Evaluations were performed at day 3, and at weeks 1, 2, 4, 6, 8, 10, and 12.

5   Results

The 28 patients were randomized to one of four treatment (or dose) groups.

The clinical response rates over time by ACR 20% criteria in each of the treatment groups is shown in
10   Table 7.

TABLE 7   Clinical Response Rates (By ACR 20% Criteria) In Patients Receiving 10 mg/kg Methotrexate

	Placebo	Dose of cA2			cA2 Treated Patients
		5 mg/kg	10 mg/kg	20 mg/kg	
Pts evaluated	7	7	7	7	21
Pts with any response	1(14.3%)	6(85.7%)	5(71.4%)	6(85.7%)	17(81.0%)
1 Week	0(0.0%)	4(57.1%)	2(28.6%)	5(71.4%)	11(52.4%)
2 Weeks	0(0.0%)	4(57.1%)	5(71.4%)	5(71.4%)	14(66.7%)
4 Weeks	1(14.3%)	3(42.9%)	5(71.4%)	5(71.4%)	13(61.9%)
6 Weeks	0(0.0%)	3(42.9%)	5(71.4%)	4(57.1%)	12(57.1%)
8 Weeks	1(14.3%)	3(42.9%)	4(57.1%)	4(57.1%)	11(52.4%)
10 Weeks	1(14.3%)	1(14.3%)	4(57.1%)	3(42.9%)	8(38.1%)
12 Weeks	1(14.3%)	2(28.6%)	4(57.1%)	3(42.9%)	9(42.9%)

Clinical benefit of cA2 treatment was evident at the first evaluation visit at one week. Although each of the 3 doses of cA2 produced clinical responses in the majority of patients treated, the duration of clinical response

5 appeared to be better sustained through 12 weeks in the groups receiving 10 or 20 mg/kg cA2. Clinical response was achieved much more frequently among patients receiving cA2 as compared to placebo. That is, 17/21 (81%) patients in the 3 cA2 groups achieved a response, compared with only

10 1/7 (14%) placebo treated patients. The magnitude of clinical response was notable. The mean tender joint count among cA2 treated patients decreased from 30.1 at baseline to 13.3 at week 12, and mean CRP decreased from 3.0 at baseline to 1.1 at week 12.

15 The duration of clinical response appeared to be dose dependent. 2/6 (33%) of the responding patients treated with 5 mg/kg cA2 sustained a response through 12 weeks of followup, compared to 7/11 (64%) of the responding patients who received 10 or 20 mg/kg. Treatment in all groups was

20 generally well tolerated.

In summary, the results of this study indicate that treatment with cA2 as adjunctive and/or concomitant therapy to methotrexate therapy is effective in the reduction of the signs and symptoms of rheumatoid arthritis in patients

25 whose disease is incompletely controlled by methotrexate. Moreover, the clinical response achieved by this approach can be sustained for more than 12 weeks after a single treatment. Accordingly, the results of this study indicate that treatment with cA2 as adjunctive and/or concomitant

30 therapy to methotrexate therapy is an important and efficacious therapeutic approach for treating RA in patients.

EXAMPLE 3 <u>Clinical Treatment of Rheumatoid Arthritis By Repeated Dose Administration of an Anti-TNF Antibody In Patients Following A Single Dose, Double-Blind, Placebo-Controlled Trial</u>

5   An open label study was conducted to evaluate the effects of repeated infusions of 10 mg/kg cA2 in combination with methotrexate, administered at a dose of 10 mg/week, in the treatment of rheumatoid arthritis in patients.

10   <u>Patients</u>

As described in Example 2, a randomized, double-blind, placebo controlled, 12 week study of cA2 was conducted in RA patients who had active disease despite receiving three months therapy with methotrexate administered at a stable
15   dose of 10 mg/wk for at least 4 weeks prior to screening.

At week 12, patients who had completed the 12 week evaluation period and had not experienced adverse events prohibiting further infusions of cA2, were offered 3 subsequent open label infusions of cA2, administered at a
20   dose of 10 mg/kg, at eight week intervals (weeks 12, 20, 28). Twenty-three (23) patients from the 12 week study were enrolled in this study.

<u>Assessments</u>

11/23 patients entering this open label study were
25   evaluated at 1 of 3 centers in the United States and followed up to 40 weeks after initial entry. Patients continued treatment with methotrexate administered at 10 mg/week throughout the study. Repeated treatments with cA2 were generally well tolerated. Three patients had
30   transient infusion related symptoms (urticaria, somnolence).

The primary measurement of clinical response was defined by the ACR preliminary definition of response

(Felson et al., Arthritis Rheumatism 38(6):727-735 (1995)).
Patients were considered to have a response if they had a
20% reduction in swollen and tender joint count, and had
experienced a 20% reduction in 3 of the 5 following

5   assessments: patient's assessment of pain (VAS), patient's
global assessment of disease activity (VAS), physician's
global assessment of disease activity (VAS), patient's
assessment of physical function (HAQ), and an acute phase
reactant (ESR). The ESR was measured at each study site

10 with a standard method (Westergen).

Results

     Of six patients who had all received cA2 during the
double-blinded study described in Example 2 and responded
through the 12 weeks of that study, four patients sustained

15 a response throughout the 40 week followup. Of the
remaining two patients, one patient is still responding
through week 28, and one patient recently entered this open
label trial. For all 4 patients completing 40 weeks of
followup and the patient at week 28, final tender joint

20 counts were 2 and swollen joint counts 1, compared to a
mean of 23 and 29, respectively, at entry into the double-
blinded study described in Example 2. For 4 of these 5
patients, ESR were 18 mm/hr and CRP 0.7, compared to a mean
of 27 and 3.9, respectively, at entry into the double-blind

25 study described in Example 2.

     Of two patients who had both received cA2 during the
double-blinded study described in Example 2 and responded
only through week 10 of that study, one patient responded
through 36 weeks and one patient is still responding

30 through week 20.

     Of three patients who did not respond during the
double-blinded study described in Example 2 (2 received
placebos, 1 received 5 mg/kg cA2), two of these patients

experienced a transient clinical response, and one patient
is still responding through week 20.

In summary, the preliminary results of this study
suggest that repeated adjunctive and/or concomitant therapy
5   with cA2, in RA patients whose disease is incompletely
controlled by methotrexate, can result in substantial
clinical improvement for a majority of the patients.
Moreover, the clinical response achieved by this approach
can be sustained for up to 40 weeks of followup.
10   Accordingly, the results of this study indicate that
repeated treatment with cA2 as adjunctive and/or
concomitant therapy to methotrexate therapy is an important
and efficacious therapeutic approach for treating RA in
patients.

15   Equivalents

Those skilled in the art will know, or be able to
ascertain, using no more than routine experimentation, many
equivalents to the specific embodiments of the invention
described herein. These and all other equivalents are
20   intended to be encompassed by the following claims.

70

The claims defining the invention are as follows:

1. A composition comprising methotrexate and a soluble TNFα receptor or functional fragment thereof.

2. A composition of claim 1, wherein the soluble TNFα receptor is selected from the group consisting of: p55 TNFα receptor and p75 TNFα receptor.

3. A composition of claim 1, wherein the soluble TNFα receptor is a TNFα receptor multimeric molecule.

4. A composition of claim 1, wherein the soluble TNFα receptor is a TNFα immunoreceptor fusion molecule.

5. A composition of claim 4, wherein the immunoreceptor fusion molecule is selected from the group consisting of p75 TNFα receptor/IgG fusion molecule and p55 TNFα receptor/IgG fusion molecule.

6. A pharmaceutical composition, substantially as hereinbefore described with reference to any one of the examples.

7. A method for treating or preventing a tumour necrosis factor-mediated disease in an individual in need thereof comprising co-administering methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 to the individual, in therapeutically effective amounts.

8. Methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 when used in treating or preventing a tumour necrosis factor-mediated disease.

9. The use of methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 for the manufacture of a medicament for treating or preventing a tumour necrosis factor-mediated disease.

10. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 7 to 9, wherein the soluble TNFα receptor and methotrexate are administered simultaneously.

11. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 7 to 9, wherein the soluble TNFα receptor and methotrexate are administered sequentially.

12. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 7 to 9, wherein the tumour necrosis factor-mediated disease is selected from the group consisting of: autoimmune disease, acute or chronic immune disease, inflammatory disease and neurodegenerative disease.

13. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 12 wherein the soluble TNFα receptor is administered in multiple doses.

14. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 13 wherein the soluble TNFα receptor is selected from the group consisting of: p55 TNFα receptor and p75 TNFα receptor.

15. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 14 wherein the soluble TNFα receptor is a TNFα receptor multimeric molecule.

16. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 14 wherein the soluble TNFα receptor is a TNFα immunoreceptor fusion molecule.

17. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 16 wherein the TNFα immunoreceptor fusion molecule is selected from the group consisting of: p55 TNFα receptor/IgG fusion protein and p75 TNFα receptor/IgG fusion protein.

18. A method for treating or preventing rheumatoid arthritis in an individual in need thereof comprising co-administering methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 to the individual, in therapeutically effective amounts.

19. Methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 when used in treating or preventing rheumatoid arthritis.

20. The use of methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 for the manufacture of a medicament for treating or preventing rheumatoid arthritis.

21. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 18 to 20, wherein the soluble TNFα receptor and methotrexate are administered simultaneously.

22. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 18 to 20, wherein the soluble TNFα receptor and methotrexate are administered sequentially.

23. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 18 to 20, wherein the soluble TNFα receptor is administered in multiple doses.

24. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 23 wherein the soluble TNFα receptor is selected from the group consisting of: p55 TNFα receptor and p75 TNFα receptor.

25. A method, methotrexate and an soluble TNFα receptor or functional fragment thereof or use of claim 24 wherein the soluble TNFα receptor is a TNFα receptor multimeric molecule.

26. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 24 wherein the soluble TNFα receptor is a TNFα immunoreceptor fusion molecule.

27. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 26 wherein the TNFα immunoreceptor fusion molecule is selected from the group consisting of: p55 TNFα receptor/IgG fusion protein and p75 TNFα receptor/IgG fusion protein.

28. A method for treating or preventing Crohn's disease in an individual in need thereof comprising co-administering methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 to the individual, in therapeutically effective amounts.

29. Methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 when used in treating or preventing Crohn's disease.

30. The use of methotrexate and a soluble TNFα receptor or functional fragment thereof or a composition of any one of claims 1 to 6 for the manufacture of a medicament for treating or preventing Crohn's disease.

31. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 28 to 30, wherein the soluble TNFα receptor and methotrexate are administered simultaneously.

32. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 28 to 30, wherein the soluble TNFα receptor and methotrexate are administered sequentially.

33. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of any one of claims 28 to 30, wherein the soluble TNFα receptor is administered in multiple doses.

34. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 33 wherein the soluble TNFα receptor is selected from the group consisting of: p55 TNFα receptor and p75 TNFα receptor.

35. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 34 wherein the soluble TNFα receptor is a TNFα receptor multimeric molecule.

36. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claims 34, wherein the soluble TNFα receptor is a TNFα immunoreceptor fusion molecule.

37. A method, methotrexate and a soluble TNFα receptor or functional fragment thereof or use of claim 34 wherein the soluble TNFα receptor is a TNFα immunoreceptor fusion molecule selected from the group consisting of: p55 TNFα receptor/IgG fusion protein and p75 TNFα receptor/IgG fusion protein.

38. Use of methotrexate and a soluble TNFα receptor or functional fragment thereof for the manufacture of coadministrable or sequentially administrable medicaments, for treating or preventing a tumour necrosis factor-mediated disease in an individual.

39. The use of claim 38, for the treatment of tumour necrosis factor-mediated diseases selected from autoimmune disease, acute or chronic immune disease, inflammatory disease and neurodegenerative disease.

40. Use of methotrexate and a soluble TNFα receptor for the manufacture of coadministrable or sequentially administrable medicaments for treating or preventing rheumatoid arthritis in an individual.

41. Use of methotrexate and a soluble TNFα receptor for the manufacture of coadministrable or sequentially administrable medicaments, for treating or preventing Crohn's disease in an individual.

42. The use of claim 38, 40 or 41, wherein the soluble TNFα receptor.

43. The use of claim 38, 40 or 41, wherein the soluble TNFα receptor is selected from the group consisting of: p55 TNFα receptor and p75 TNFα receptor.

44. The use of claim 38, 40 or 41, wherein the soluble TNFα receptor is a TNFα receptor multimeric molecule.

45. The use of claim 38, 40 or 41, wherein the soluble TNFα receptor is a TNFα immunoreceptor fusion molecule.

46. A method for treating or preventing a tumour necrosis factor-mediated disease in an individual in need thereof comprising co-administering to the individual methotrexate and a tumour necrosis factor antagonist, wherein the tumour necrosis factor antagonist prevents or inhibits TNF synthesis, TNF release or TNF receptor signalling.

47.   Methotrexate and a tumour necrosis factor antagonist when used in treating or preventing a tumour necrosis factor-mediated disease, wherein the tumour necrosis factor antagonist prevents or inhibits TNF synthesis, TNF release or TNF receptor signalling.

48.   Use of methotrexate and a tumour necrosis factor antagonist for the manufacture of a medicament for treating or preventing a tumour necrosis factor-mediated disease, wherein the tumour necrosis factor antagonist prevents or inhibits TNF synthesis, TNF release or TNF receptor signalling.

49.   A method, methotrexate and a tumour necrosis factor antagonist or use of any one of claims 46 to 48, wherein the tumour necrosis factor antagonist is a phosphodiesterase inhibitor.

50.   A method, methotrexate and a tumour necrosis factor antagonist or use of claim 49, wherein the phosphodiesterase inhibitor is selected from the group consisting of: pentoxifylline and rolipram.

51.   A method, methotrexate and a tumour necrosis factor antagonist or use of any one of claims 46 to 48, wherein the tumour necrosis factor antagonist is selected from the groups consisting of A2b zadenosine receptor enhancer and A2b adenosine receptor agonist.

52.   A method, methotrexate and a tumour necrosis factor antagonist or use of claim 51, wherein the tumour necrosis factor antagonist is selected from the group consisting of: 5'-(N-cyclopropyl)-carboxamidoadenosine, 5'-N-ethylcarboxamidoadenosine, cyclohexyladenosine and R-N^6-phenyl-2-propyladenosine.

53.   Use of methotrexate and a tumour necrosis factor antagonist for the manufacture of medicaments for treating or preventing a tumour necrosis factor-mediated disease in an individual in need thereof, wherein the tumour necrosis factor antagonist prevents or inhibits TNF synthesis, TNF release or TNF receptor signalling.

54.   Use of claim 53, wherein the tumour necrosis factor antagonist is a phosphodiesterase inhibitor.

55.   Use of claim 54, the phosphodiesterase inhibitor is selected from the group consisting of: pentoxifylline and rolipram.

56.   Use of claim 53, wherein the tumour necrosis factor antagonist is selected from the group consisting of: A2b adenosine receptor enhancer and A2b adenosine receptor agonist.

57.   Use of claim 56, wherein the tumour necrosis factor antagonist is selected from the group consisting of: 5'-(N-cyclopropyl)-carboxamidoadenosine, 5'-N-ethylcarboxamidoadenosine, cyclohexyladenosine and R-N^6-phenyl-2-propyladenosine.

58. A composition comprising methotrexate and an anti-tumour necrosis factor antibody or fragment thereof.

59. A composition of claim 58, wherein the anti-tumour necrosis factor antibody is a chimeric antibody.

60. A composition of claim 58, wherein the chimeric antibody binds to one or more amino acids of hTNFα selected from the group consisting of about 87-108 and about 59-80.

61. A composition of claim 58, wherein the chimeric antibody binds to the epitope of cA2.

62. A composition of claim 61, wherein the chimeric antibody is cA2.

63. A pharmaceutical composition, substantially as hereinbefore described with reference to any one of the examples.

64. A method for treating or preventing a tumour necrosis factor-mediated disease in an individual in need thereof comprising co-administering methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 to the individual, in therapeutically effective amounts.

65. Methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 when used in treating or preventing a tumour necrosis factor-mediated disease.

66. The use of methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 for the manufacture of a medicament for treating or preventing a tumour necrosis factor-mediated disease.

67. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 64 to 66, wherein the anti-tumour necrosis factor antibody and methotrexate are administered simultaneously.

68. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 64 to 66, wherein the anti-tumour necrosis factor antibody and methotrexate are administered sequentially.

69. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 64 to 66, wherein the tumour necrosis factor-mediated disease is selected from the group consisting of: autoimmune disease, acute or chronic immune disease, inflammatory disease and neurodegenerative disease.

70. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 69 wherein the anti-tumour necrosis factor antibody is administered in multiple doses.

71. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 70 wherein the anti-tumour necrosis factor antibody is a chimeric antibody.

72. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 71 wherein the chimeric antibody binds to one or more amino acids of hTNFα selected from the group consisting of about 87-108 and about 59-80.

73. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 71 wherein the chimeric antibody binds to the epitope of cA2.

74. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 73 wherein the chimeric antibody is cA2.

75. A method for treating or preventing rheumatoid arthritis in an individual in need thereof comprising co-administering methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 1 to 6 to the individual, in therapeutically effective amounts.

76. Methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 when used in treating or preventing rheumatoid arthritis.

77. The use of methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 for the manufacture of a medicament for treating or preventing rheumatoid arthritis.

78. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 75 to 77, wherein the anti-tumour necrosis factor antibody and methotrexate are administered simultaneously.

79. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 75 to 77, wherein the anti-tumour necrosis factor antibody and methotrexate are administered sequentially.

80. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 75 to 77, wherein the anti-tumour necrosis factor antibody is administered in multiple doses.

81. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 80 wherein the anti-tumour necrosis factor antibody is a chimeric antibody.

82. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 81 wherein the chimeric antibody binds to one or more amino acids of hTNFα selected from the group consisting of about 87-108 and about 59-80.

83. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 81 wherein the chimeric antibody binds to the epitope of cA2.

84. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 83 wherein the chimeric antibody is cA2.

85. A method for treating or preventing Crohn's disease in an individual in need thereof comprising co-administering methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 1 to 6 to the individual, in therapeutically effective amounts.

86. Methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 when used in treating or preventing Crohn's disease.

87. The use of methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or a composition of any one of claims 58 to 63 for the manufacture of a medicament for treating or preventing Crohn's disease.

88. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 85 to 87, wherein the anti-tumour necrosis factor antibody and methotrexate are administered simultaneously.

89. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 85 to 87, wherein the anti-tumour necrosis factor antibody and methotrexate are administered sequentially.

90. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of any one of claims 85 to 87, wherein the anti-tumour necrosis factor antibody is administered in multiple doses.

91. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 90 wherein the anti-tumour necrosis factor antibody is a chimeric antibody.

92. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 91 wherein the chimeric antibody binds to one or more amino acids of hTNFα selected from the group consisting of about 87-108 and about 59-80.

93. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 91, wherein the chimeric antibody binds to the epitope of cA2.

94. A method, methotrexate and an anti-tumour necrosis factor antibody or fragment thereof or use of claim 91 wherein the chimeric antibody is cA2.

95. A method for treating or preventing a tumour necrosis factor-mediated disease in an individual in need thereof comprising co-administering methotrexate and a tumour necrosis factor antagonist to the individual, in therapeutically effective amounts.

96. Methotrexate and a tumour necrosis factor antagonist when used in treating or preventing a tumour necrosis factor-mediated disease.

97. The use of methotrexate and a tumour necrosis factor antagonist for the manufacture of a medicament for treating or preventing a tumour necrosis factor-mediated disease.

98. Use of methotrexate and an anti-tumour necrosis factor antibody or fragment thereof for the manufacture of coadministrable or sequentially administrable medicaments, for treating or preventing a tumour necrosis factor-mediated disease in an individual.

99. The use of claim 98, for the treatment of tumour necrosis factor-mediated diseases selected from autoimmune disease, acute or chronic immune disease, inflammatory disease and neurodegenerative disease.

100. Use of methotrexate and ant-tumour necrosis factor antibody for the manufacture of coadministrable or sequentially administrable medicaments for treating or preventing rheumatoid arthritis in an individual.

101. Use of methotrexate and an anti-tumour necrosis factor antibody for the manufacture of coadministrable or sequentially administrable medicaments, for treating or preventing Crohn's disease in an individual.

102. The use of claim 98, 100 or 101, wherein the anti-tumour necrosis factor antibody is a chimeric antibody.

103. The use of claim 98, 100 or 101, wherein the chimeric antibody binds to one or more amino acids of hTNFα selected from the group consisting of about 87-108 and about 59-80.

104. The use of claim 98, 100 or 101, wherein the chimeric antibody binds to the epitope of cA2.

105. The use of claim 98, 100 or 101, wherein the chimeric antibody is cA2.

106. Use of methotrexate and a tumour necrosis factor antagonist for the manufacture of medicaments for treating or preventing a tumour necrosis factor-mediated disease in an individual in need thereof.

Dated 4 August, 2000
The Kennedy Institute of Rheumatology

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

1 mg/kg

Swollen Joint Count

—O— cA2 – MTX-
—●— cA2 – MTX+
—□— Placebo – MTX+

Weeks	0	2		6		10		14		18		22		28	
(n), cA2 – MTX-	15	15	15	15	9	8	8	8	7	5	4	4		4
(n), cA2 – MTX+	14	14	14	14	14	14	13	13	11	11	10	9		9
(n), Placebo – MTX+	14	13	8	8	6	6	6	6	5	5	4	3		3

FIGURE 1A

3 mg/kg

Swollen Joint Count

—O— cA2 – MTX-
—●— cA2 – MTX+

Weeks	0	2		6		10		14		18		22		26
(n), cA2 – MTX-	14	14	14	13	13	13	13	12	11	11	10	9		9
(n), cA2 – MTX+	15	15	15	15	15	15	15	15	15	14	13	13		13

FIGURE 1B

10 mg/kg

Swollen Joint Count

—O— cA2 – MTX-
—●— cA2 – MTX+

Weeks	0	2		6		10		14		18		22		26
(n), cA2 – MTX-	15	15	15	14	12	12	12	12	12	11	11	10		9
(n), cA2 – MTX+	14	14	14	14	13	13	12	12	12	12	12	12		11

FIGURE 1C

1 mg/kg

Tender Joint Count

—○— cA2 – MTX-
—●— cA2 – MTX+
—□— Placebo – MTX+

Weeks	0	2		6		10		14		18		22		26	
(n), cA2 – MTX-	15	15	15	9	8	8	8	7	5	4	4		4	
(n), cA2 – MTX+	14	14	14	14	14	13	13	11	11	10	9		9	
(n), Placebo – MTX+	14	13	8	8	6	6	6	5	5	4	3		3	

FIGURE 2A

3 mg/kg

Tender Joint Count

—○— cA2 – MTX-
—●— cA2 – MTX+

Weeks	0	2		6		10		14		18		22		26
(n), cA2 – MTX-	14	14	14	13	13	13	13	12	11	11	10		9
(n), cA2 – MTX+	15	15	15	15	15	15	15	15	15	14	13		13

FIGURE 2B

10 mg/kg

Tender Joint Count

—○— cA2 – MTX-
—●— cA2 – MTX+

Weeks	0	2		6		10		14		18		22		26
(n), cA2 – MTX-	15	15	15	14	12	13	12	12	12	11	11		9
(n), cA2 – MTX+	14	14	14	14	13	13	12	12	12	12	12		11

FIGURE 2C

1 mg/kg

Physician's Global Disease Assessment (VAS – 0-10 cm)

cA2 – MTX-
cA2 – MTX+
Placebo – MTX+

Weeks

0	2		6		10		14		18		22	26

(n). cA2 – MTX-	15	15	15	15	9	8	8	8	7	5	4	4	4
(n). cA2 – MTX+	14	14	14	14	14	6	13	6	11	11	10	9	9
(n). Placebo – MTX+	14	13	8	8	6	6	6	6	5	5	4	3	3

FIGURE 3A

3 mg/kg

Physician's Global Disease Assessment (VAS – 0-10 cm)

cA2 – MTX-
cA2 – MTX+

Weeks

0	2		6		10		14		18		22	26

| (n). cA2 – MTX- | 14 | 14 | 14 | 13 | 13 | 13 | 13 | 12 | 11 | 11 | 10 | 9 | 9 |
| (n). cA2 – MTX+ | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 14 | 13 | 13 | 13 |

FIGURE 3B

10 mg/kg

Physician's Global Disease Assessment (VAS – 0-10 cm)

cA2 – MTX-
cA2 – MTX+

Weeks

0	2		6		10		14		18		22	26

| (n). cA2 – MTX- | 15 | 15 | 15 | 14 | 12 | 12 | 12 | 12 | 12 | 11 | 11 | 10 | 9 |
| (n). cA2 – MTX+ | 14 | 14 | 14 | 14 | 13 | 13 | 12 | 12 | 12 | 12 | 12 | | 11 |

FIGURE 3C

1 mg/kg

													Weeks
0		2		6		10		14		18		22	26
15	15	15	15	9	8	8	8	7	5	4	4		4	(n), cA2 – MTX-
14	14	14	14	14	9	14	13	13	11	11	10		9	(n), cA2 – MTX+
14	13	8	8	6	6	6	6	5	5	4	3		3	(n), Placebo – MTX+

FIGURE 4A

3 mg/kg

													Weeks
0		2		6		10		14		18		22	26
14	14	14	13	13	13	13	12	11	11	10	9		9	(n), cA2 – MTX-
15	15	15	15	15	15	15	15	15	14	13	13		13	(n), cA2 – MTX+

FIGURE 4B

10 mg/kg

													Weeks
0		2		6		10		14		18		22	26
15	15	15	14	12	12	12	12	12	11	11	10		9	(n), cA2 – MTX-
14	14	14	14	13	13	12	12	12	12	12	12		11	(n), cA2 – MTX+

FIGURE 4C

1 mg/kg

FIGURE 5A

3 mg/kg

FIGURE 5B

10 mg/kg

FIGURE 5C

1 mg/kg

Health Assessment Questionnaire (y-axis: 2.0, 1.5, 1.0, 0.5, 0.0)

													Weeks
0	2		6		10		14		18		22		26
15	15	15	15	9	8	8	8	7	5		4		4	(n), cA2 – MTX-
14	14	14	14	14	14	13	13	11	11	5	4		4	(n), cA2 – MTX+
14	13	8	8	6	6	6	6	5	5	4	3		3	(n), Placebo – MTX+

cA2 – MTX-
cA2 – MTX+
Placebo – MTX+

FIGURE 6A

3 mg/kg

Health Assessment Questionnaire (y-axis: 2.5, 2.0, 1.5, 1.0, 0.5)

													Weeks
0	2		6		10		14		18		22		26
14	14	14	13	13	13	13	12	11	11	10	9		9	(n), cA2 – MTX-
15	15	15	15	15	15	15	15	15	14	13	13		13	(n), cA2 – MTX+

cA2 – MTX-
cA2 – MTX+

FIGURE 6B

10 mg/kg

Health Assessment Questionnaire (y-axis: 2.5, 2.0, 1.5, 1.0, 0.5)

													Weeks
0	2		6		10		14		18		22		26
15	15	15	14	12	12	12	12	12	11	11	10		9	(n), cA2 – MTX-
14	14	14	14	13	13	12	12	12	12	12	12		11	(n), cA2 – MTX+

cA2 – MTX-
cA2 – MTX+
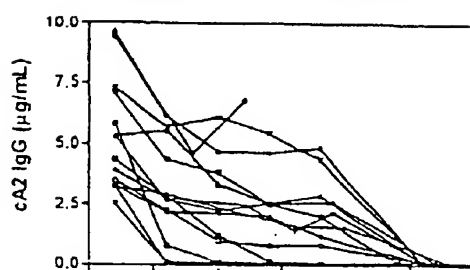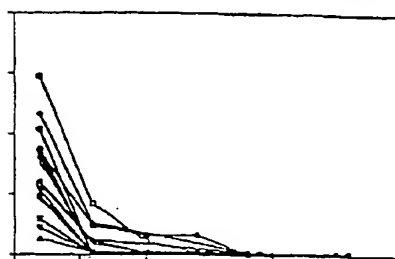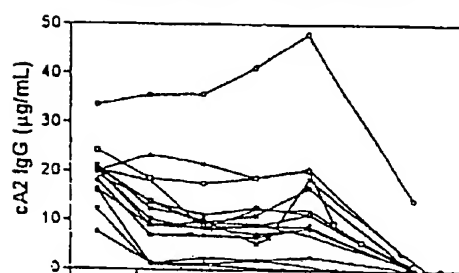
FIGURE 6C

1 mg/kg cA2 + methotrexate

FIGURE 7A

1 mg/kg cA2, no methotrexate

FIGURE 7B

3 mg/kg cA2 + methotrexate

FIGURE 7C

3 mg/kg cA2, no methotrexate

FIGURE 7D

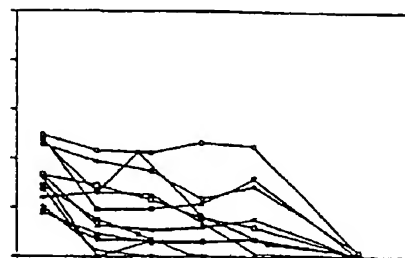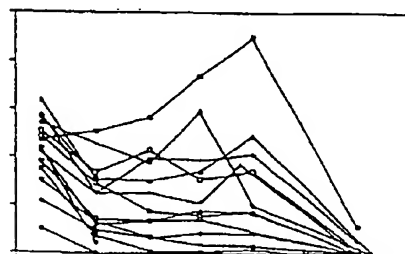10 mg/kg cA2 + methotrexate

Weeks Post Infusion #1

FIGURE 7E

10 mg/kg cA2, no methotrexate

Weeks Post Infusion #1

FIGURE 7F

Serum cA2 Concentrations

FIGURE 8A

FIGURE 8B